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Abstract Book

Combined targeting of growth-promoting genes with antisense oligonucleotides in human colorectal cancer cells: Chemo-sensitization potential

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Background: Aberrant expression of growth-promoting genes contributes to the growth advantage of tumor cells, targeting such genes with phosphorothioated antisense oligonucleotides, AS[S]ODNs, might therefore be useful in controlling the abnormal proliferation of cancer cells. To explore the potential of combination antigene therapy in human colorectal cancer cells, we have examined the *in vitro* effects of AS[S]ODNs targeting c-myb, c-myc and cdc2 in human colorectal cancer cell lines.
Method: Cancer cells were treated with c-myb-, c-myc- or cdc2-AS[S]ODNs individually or in combination. The effects of growth promoting-gene AS[S]ODNs on mRNA and protein expression were determined by RT-PCR and blot analysis. The effects of these combinations on cell growth, chemo-sensitization, apoptosis and genes controlling cell growth and apoptosis were monitored by MTT assay, DNA fragmentation and Real-time RT-PCR.
Results: mRNA and protein expression were dramatically reduced after treatment with c-myb-, c-myc-, or cdc2-AS[S]ODNs. Combined targeting of c-myb/c-myc and c-myb/cdc2 had much higher dose and time dependent synergistic growth inhibitory effects; 5-100% and 5-95%, respectively; compared to single antigen therapy (5-55%). Combined targeting of c-myc / cdc2 also produced greater dose and time dependent additive or synergistic growth inhibitory effects (10-100%) compared to single antigene therapy (5-60%). The combined targeting of c-myb / c-myc / cdc2 exhibited much higher growth inhibitory effects (10-90%) compared to single antigene therapy (20-50%). Combined targeting of c-myb / c-myc / cdc2 produced marked inducing effects on both apoptosis and chemo-sensitization to taxol, 5FU, doxorubicin and vinblastine. Real-time RT-PCR indicated down-regulation of mRNAs of cdk1, cyclin B1, cdk2, cyclin E1, cdk4, cyclin D1, BcL2 and BclxL and up-regulation of mRNAs of p21, Bax and caspase.
Conclusion: Our study suggests that combination antigene therapy targeting c-myb, c-myc and cdc2 can inhibit human colorectal cancer cell proliferation more effectively than monogene therapy by blocking the cell cycle and inducing apoptosis. Combination antigene therapy is thus a promising approach for cancer therapy.

Introduction of supercritical fluid extraction as a new sample-preparation procedure for isolation and identification of a pharmaceutical from biological fluids: Application to disposition kinetics

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Background: Since its commercial development in the early 1990s, supercritical fluid extraction (SFE) has attracted considerable attention as a sample-preparation procedure. However, other different sample preparation procedures, including precipitation, liquid- and/or solid-phase extraction in biological fluids, also remain in use. Aims: In this investigation, SFE was introduced to isolate and identify orbifloxacin (OBFX) from plasma and milk of lactating does.
Methods: Four parameters, including the temperature and the pressure of supercritical fluid, modifier ratios, and dynamic extraction time, were evaluated and optimized to obtain the best yield of the analyte from the biological fluids. Determinations of the OBFX in the extracts were carried out using high-performance liquid chromatography coupled with fluorescence detector (HPLC-FLD). The linearity of the calibration curves as well as the instrument limit of detection /limit of quantitation (LOD/LOQ) were evaluated.
Results: The optimum conditions of the extraction process that yielded the maximum analyte extraction efficiencies were 150°C vs. 60°C, 250 kg/cm^2, 30% vs. 35% methanol, and 40 min vs. 20 min, for plasma and milk, respectively. Good linearity (at least $r^2 \geq 0.999$) of the calibration curves was obtained over the range from 0.2 to 0.01 µg/mL. The method showed good a recovery rate (74.2–127.73%) and precision (expressed as relative standard deviations (RSDs) 1.64–20%). The instrumental LOD and LOQ values were 0.004 µg/mL vs. 0.01 µg/mL or 0.006 µg/mL vs. 0.02 µg/ L, for plasma and milk, respectively. The method was successfully applied to estimate the pharmacokinetic variables of orbifloxacin in lactating does.
Conclusions: To the best of our knowledge, this is the first time that SFE has been applied to isolate an antimicrobial agent from biological fluids. This method is promising for clinical applications and for pharmacokinetic studies of various pharmaceuticals in biological fluids.

Misoprostol and Postpartum Hemorrhage

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Abstract:
Every year more than half a million women lose their lives because of complications of pregnancy and childbirth. Postpartum hemorrhage is the leading cause of death especially in developing countries. Strategies have been developed to prevent and treat postpartum hemorrhage. One of these is the use of uterotonic drugs to prevent and treat atonic postpartum hemorrhage. Misoprostol (prostaglandin E1 analogue) has been emerged as an uterotonic drug with special advantages; being heat stable, can be used orally and rather cheap. So, it can be considered as a potential "Magic Bullet" in the management of postpartum hemorrhage. Because of this potentiality, World Health Organization has been conducting big multicentre randomized clinical trials to evaluate efficacy and safety of misoprostol in the prevention and treatment of postpartum hemorrhage. The findings and implications of these trials will be presented and discussed.

Magic bullets in prostate cancer

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It has been 100 years since Paul Ehrlich popularized the concept of a "magic bullet" during his 1908 Nobel prize lecture. Scientists have since keenly embarked on the search for these magic bullets. In the UK prostate cancer is the most common cancer diagnosed in men and the second leading cause of cancer death in men (1). Consequently a magic bullet is highly desired by patients and urologists alike.
The first bullet comprises hormonal therapy resulting in androgen ablation, which represent the mainstay of treatment of metastatic prostate cancer. LHRH analogues can be implanted into patients where they cause a negative feedback effect, resulting in a decrease in LHRH production from the anterior pituitary and a subsequent reduction in testicular testosterone production. Degarelix, an LHRH antagonist has an immediate onset of action suppressing LHRH and testosterone, thus avoiding tumour flare. Studies suggest a PSA decrease by 97-98% after 1 year and the median time to 90% reduction in PSA was 8 weeks (2).
Brachytherapy is ultrasound guided implanation of radioactive seeds into the prostate. Its popularity has increased as the use of transrectal ultrasound (TRUS) makes it easier to accurately direct the magic bullet. Patient selection is a key. In this scenario brachytherapy achieves a 12- year progression free survival of 66% (3).
Heat effects are attractive. HIFU is a bullet where prostate tissue is heated to the point of coagulative necrosis using high-energy ultrasound allowing the selective destruction of tissues. Long-term results from clinical trials are awaited. Freezing or cryotherapy of the prostate cancer has increased over the past few years, especially with the improvement in cryotechnology. Multiple hollow core probes are placed percutaneously under TRUS guidance. This causes cell destruction at ˜20 - ˜ 40ºc, usually achieved by applying two cycles of freeze thaw. 96% of men with localised disease achieve a PSA of <0.2ng/ml within 6 months. Again long term results are awaited.
Finally, Docetaxel, chemotherapy agent, is a magic bullet that may be used to treat androgen-independent prostate cancer, with promising results (4).

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All abstracts are listed in alphabetical order of the presenting author.

Non-Systemic Delivery of Ocular Brimonidine to the Brain: Extending the Therapeutic Benefits of Brimonidine to the CNS

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Background. Despite recent advancements in neuroscience, and ever-emerging novelties in the field of drug delivery, the blood brain barrier is still considered a major obstacle that critically limits the delivery to the brain of hydrophilic drugs or those with modest or weak hydrophobic character. Brimonidine, a selective alpha-2 agonist, is a widely used ocular hypotensive agent with promising merits as neuroprotectant.
Aim. To demonstrate the efficient delivery to the brain of Brimonidine following ocular administration and to elucidate the route of non-systemic drug flow along the eye-brain axis.
Methods. Brimonidine was used as a probe to study the eye and brain pharmacokinetics following topical ocular administration of one droplet of the radio-labeled drug. Animals were sacrificed at different time points following the administration of single 0.2% ^{3}H-Brimonidine droplet to the rabbit eye. Brain and eyes were dissected and selected brain and eye tissues, as well as systemic blood samples, were processed for detecting the concentration of radio-labeled Brimonidine. Fluorescent-labeled Dextran with molecular weight of 40 kDa was used as a probe to elucidate the route of the non-systemically mediated eye-to-brain drug flow following periocular administration.
Results. Brimonidine accumulation in studied intracranial tissues was significant already at 5 minutes following single ocular administration while the detected Brimonidine levels in the systemic blood were very low at all time points. Evaluation by fluorescent microscopy showed that 40 kDa fluorescent-labeled Dextran that was administered to the periocular space didn't permeate the ocular barriers and was flowing through veins that drain the eye and orbital tissues towards the intracranial cavernous sinus. Tissue sections that included the eye and its major vessels showed extensive accumulation in veins but with no signal of the fluorescented probe inside the ciliary artery, which corresponds to non-significant flow of the probe through the systemic circulation.
Conclusion. Brimonidine, a non-toxic agent to the eye with well appreciated therapeutic merits, can accumulate in intracranial tissues at significant concentrations already at few minutes after simple ocular administration with very low systemic accumulation. Our results suggest an efficient method for the extension of Brimonidine therapeutic merits to the brain while avoiding systemic side effects.

A Role of Lysosomal Phospholipase A2 in Cationic Amphiphilic Drug-induced Phospholipidosis

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Background: Many of cationic amphiphilic drugs (CADs) developed as therapeutic drugs, including those with anti-inflammatory, antineoplastic and antiangiogenic properties, and have been known to induce phospholipidosis in human and other mammalian tissues. Generally, CADs tend to accumulate in lysosomes and to inhibit lysosomal enzyme activities. Recently, it was found that the alveolar macrophages (AMs) prepared from lysosomal phospholipase A2 (LPLA2) deficient mice suffer from cellular phospholipid accumulation and phospholipidosis. This phenotype suggests a potential connection between CAD-induced phospholipidosis and LPLA2. The study on the reaction properties of LPLA2 could find a clue to understand the molecular mechanism of CAD-induced phospholipidosis. LPLA2 shows an increased activity towards zwitterionic phosphatidylcholine liposomes containing negatively charged lipids under acidic conditions. In the present study, the effect of negatively charged lipid on LPLA2 activity was investigated.
Methods: Purified recombinant mouse LPLA2 was used as the source of LPLA2. Sulfatide, a lipid that is resistant to LPLA2, was chosen as a negatively charged lipid.
Results: Sulfatide incorporated into 1, 2-dioleoyl-glycero-3-phosphocholine (DOPC) liposomes enhanced LPLA2 activity under acidic conditions. The enhancement by sulfatide was linear until 10% molar ratio of sulfatide to DOPC and weakened by the addition of NaCl in the reaction mixture. Amiodarone (AMD), a cationic amphiphilic drug that interacts with negatively charged lipids, reduced the LPLA2 activity in a concentration dependent manner. In addition, the co-sedimentation of LPLA2 with negatively charged liposomes occurred in centrifuge at 150,000 *g* and was markedly reduced by the addition of NaCl or AMD in the reaction mixture. These results indicate that LPLA2 adsorption to the negatively charged lipid membrane surface through an electrostatic attraction enhances the rate of phospholipid hydrolysis by LPLA2 at lipid-water interfaces.
Conclusions: CAD-induced cellular phospholipidosis is linked to the impairment of phospholipid catabolism by inhibition of the binding of LPLA2 to the membrane treated with CDA.

Effect Of Flunixin On Enrofloxacin Clearance And Steady-State Serum Concentrations In Calves

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Background: To evaluate whether the concomitant administration of flunixin may alter enrofloxacin pharmacokinetic parameters and the hepato-renal activities.
Methods:: Ten clinically healthy, Freisian calves weighting 200-250 kg and 5-7 months old were used. They were divided up into two groups. First group was injected a single dose of enrofloxacin 2.5 mg/kg of body weight (BW) intravenously (IV). Second group was injected the same dose intramuscularly (IM). After 1 month washout period, each of the 10 animals was given flunixin (IM) at a dose 2.2 mg/kg of BW one-hour prior to with the injection of enrofloxacin in a dose of 2.5 mg/kg BW in calves of the first group IV. or the IM injection in the second group.
Results: Co-administration of flunixin with IV injection of enrofloxacin reduced the volume of distribution at steady state $\mathbf{V_{d(SS)}}$ and total body clearance $\mathbf{(Cl_{tot})}$ by 33.9% and 30% respectively. After IM injection of enrofloxacin, the elimination half-life ($t_{1/2el}$) and mean residence time (MRT) were shorter in the flunixin-medicated calves.

Parameters	Unit	Enrofloxacin alone	Enrofloxacin + flunixin
I/V			
$V_{d(SS)}$	L kg^{-1}	2.24 ± 0.17	1.48 ± 0.04**
Cl_B	ml/min/kg	1.73 ± 0.10	1.21 ± 0.04**
$AUC_{0-?}$	μg.h/mL	1.58 ± 0.13	2.17 ± 0.06**
I/M			
C_{max}	μg/mL	0.31 ± 0.01	0.26 ± 0.01**
T_{max}	h	0.94 ± 0.02	0.69 ± 0.05**
MRT	h	3.62 ± 0.24	2.23 ± 0.29**
($t_{1/2el}$)	h	2.24 ± 0.14	1.45 ± 0.11**
$AUC_{0-?}$	μg.h/mL	1.42 ± 0.11	0.83 ± 0.03***

C_{max}: maximum plasma or milk concentration; T_{max}: time to peak concentration; $AUC_{0-\infty}$: area under the curve from zero to infinity by the trapezoidal integral; **P<0.01; ***P<0.001

Conclusions: Concomitant administration of flunixin with enrofloxacin induced significant alterations in pharmacokinetic parameters and hepatic-renal functions in calves. Therefore, concurrent administration of flunixin with enrofloxacin should be avoided.

Selenium Derivatives as Cancer Preventive Agents

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Background: The role of selenium in the prevention of cancer has been recently established by laboratory experiments, clinical trials, and epidemiological data. Most of the effects are related to the function of selenium in antioxidant enzyme systems. Animal data, epidemiological data, and intervention trials have shown a clear role for selenium derivatives in both prevention of specific cancers and antitumorigenic effects in postinitiation phases of cancer.
Methods: Selenazolidine prodrugs (SCA) of selenocysteine were synthesized by the reaction of selenocysteine with the appropriate carbonyl derivative. Male CF1 mice were treated daily for 7 days with equi-selenium (1.25 mg Se/kg) doses of each agent, by either the intraperitoneal (ip) or intragastric (ig). route and the effects compared with those of selenocystine. Hepatic parameters were determined 24 hours after the last dose.The efficacy of SCA in reducing NNK [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone]-induced lung adenomas in female A/J mice, a model for tobacco-related lung tumorigenesis, has been investigated with selenazolidines in the diet for 1 month prior to carcinogen administration and during the subsequent 4 months of tumor development.
Results: In general, few significant ($p < 0.05$) changes were seen with ig as compared to ip administration. 2-butylSCA, 2-cyclohexylSCA, 2-phenylSCA and 2-oxoSCA were chemopreventive, significantly reducing mean lung tumor numbers from the 10.9 of unsupplemented controls to 4.7, 5.3, 2.8 and 4.7, respectively. When selenazolidine supplementation began three days after carcinogen administration (i.e., post-initiation), 2-butylSCA, 2-cyclohexylSCA, and 2-oxoSCA were chemopreventive. In both regimens, selenocystine was also chemopreventive. Both 2-butylSCA and 2-phenylSCA retained their chemopreventive activity (44% and 40% tumor number reduction, respectively), when the supplementation was shortened and restricted to a pre-initiation period (days −9 to−2).
Conclusions: Although this study has not identified the mechanism, it firmly establishes that 2-substituted selenazolidine- 4(*R*)-carboxylic acids possess chemopreventive activity against NNK-induced lung tumors in a murine model. Dependent on the nature of the 2-substituent, the chemopreventive activity can arise from changes elicited in the post-initiation period, similar to selenocystine, or in the pre-initiation period.

Xenon: a magic bullet for the treatment of acute brain ischemia?

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Background: As a general consensus, the noble and remarkably safe anesthetic gas xenon, which possesses low-affinity antagonistic properties at the NMDA receptor, has been clearly demonstrated as a promising preclinical neuroprotective agent for the treatment of acute cerebral ischemia (and other hypoxic-ischemic insults), with no proven adverse side effects when used at subanesthetic concentrations. However, surprisingly, possible interactions between xenon and tissue-type plasminogen activator (t-PA), a serine protease with thrombolytic activity that is the only drug approved for the treatment of acute brain ischemia, have never been investigated.
Methods: We review *in vitro* and *in vivo* unpublished and published structural, electrophysiological, electrochemical, biochemical, and pharmacological data on the mechanisms of action and the neuroprotective effects of xenon, and its interactions with t-PA.
Results: We show that xenon would produce its antagonistic action at the NMDA receptor by binding on a specific hydrophobic pocket that may be located on the NR1 subunit. In addition, we also show that xenon has exceptional neuroprotective properties that take place within a limited but interesting therapeutic window following ischemia onset. Finally, we show *de novo* that xenon at concentrations higher than 25 vol% reduces the thrombolytic and proteolytic properties of t-PA both in vitro and in vivo in rats subjected to a thrombo-embolic model of middle cerebral artery by interacting directly with the catalytic site of t-PA.
Conclusions: Overall, these data indicate that xenon should be used for the treatment of acute ischemic stroke according to a well-defined therapeutic sequence. We propose that xenon at concentrations higher than 25 vol% should not be administered in patients suffering ischemia due to the risk of inhibiting the benefits of t-PA-induced thrombolysis or possible endogenous fibrinolysis in patients who cannot be treated with t-PA. Then, once blood flow would be restored spontaneously or by t-PA, xenon could be used at higher subanesthetic doses, if necessary, to increase neuroprotection and to reduce the risk of adverse proteolytic side-effect of t-PA therapy that is hemorrhaging.

Central Nervous Systems involvement in dogs naturally infected by *Leishmania chagasi/infantum*

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Blood brain barrier (BBB) was described by Paul Ehrlich when he injected dyes in the blood stream and observed that all tissues stained, except the organs of central nervous system (CNS). This barrier is very efficient in controlling substances that could cause damage to the brain. However, several pathogens are able to cross it. In order to demonstrate that *Leishmania chagasi/ infantum* is also capable to produce lesions in CNS, the brain of thirty dogs naturally infected by the protozoan were studied. The samples were submitted to histopathological, imunohistochemical exams and Polimerase Chain Reaction (PCR). Histopathological analysis revealed that the amastigotes forms were associated to an inflammatory reaction. It was observed mainly in the cerebellum and thalamus. Both PCR and immunohistochemistry confirmed the presence of the parasite in 20% of the examined brains. Previous studies in our laboratory evidenced that mice experimentally infected by *L. amazonensis* also developed inflammatory infiltrates composed by eosinophils and parasitized macrophages. Mast cells were frequently observed. Both studies demonstrated that *Leishmania* is able to change the permeability of BBB leading to lesions. Although, the brain is considered a healthy place immunologically privileged several pathogens can cross this barrier and cause injury. Different hypotheses have been postulated to explain how the efficient BBB is impaired. In accordance to Persidsky et al. (2006), it occurs during inflammatory process due to disruption of junction complexes between brain microvascular endothelial cells with subsequent formation of a paracellular route that facilitates entry of leukocytes into cerebral parenchyma. We suppose that Leishmania reached brain of mice and canine through immature monocytes.

New Silver Compounds as Wound Healing Material Problems and Opportunities

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Wounds are a major medical problem affecting about 10% of all hospitalized patients and costing more than $5 billion US$ per year in the USA alone.[1] The major problem, especially with large wounds, such as burns, and chronical wounds, is the risk of infection. And that this not a mere inconvenience, event today infected wounds may be lethal to hospitalized patients. The antibacterial properties of silver and its low toxicity, combined with the ease of topical applications and surface coatings using the metal and its compounds has led to a revival of silver use in medicine.[2] Silver containing wound dressings are today based on mainly silver or simple silver salts such as the nitrate or the sulphate. The only silver coordination compound used to any extent is the $[Ag(sulfadiazine)]_n$ coordination polymer patented in 1973, a prescription drug in the US and sold under trade names such as Silvazine in preparations as a 1% cream.
In this contribution we will discuss some problems affecting different types of silver therapy, i.e. *silver resistant bacteria* (contrary to popular belief this does exist and may be a growing problem), *coupling of silver and antibiotic resistance* (remains to be proven, but a link exists), *in vitro and in vivo studies* (difficult to extrapolate and documented effect of existing preparations not always good).
We will also examine the opportunities that new coordination compounds may give in this respect and what grounds there are to expect that other species other than just the "naked" Ag^+ ion could be the active antimicrobial agents. New silver complexes were synthesized by direct mixing of aqueous solutions of silver nitrate and ethanolic solutions of the corresponding ligand. Also solid state techniques were employed to synthesis $[Ag(2\text{-aminopyridine})_3](NO_3)$ and $[Ag_3(2\text{-aminopyridine})_4(NO_3)_2](NO_3)$. Further synthesis and structural details are described elsewhere.[3] UV- as well as ^{1}H-NMR titrations were carried out to investigate the structural behavior and stability at different ratios. Antibacterial activity of the complexes was determined according to the recommendations of NCCLS (1999) for the use of a broth microdilution method. The activity (1/MIC) of some Ag(I) nicotinate and iso-nicotinate compounds compared to silversulfadiazine against some clinically isolated multi-resistant bacteria.[3]

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PKC-ι inhibition by ICA-1 reduces cell proliferation in Neuroblastoma

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Abstract

Neuroblastomas are highly lethal tumors and 85% of cerebral neuroblastoma occurs in children and 15% in adults. Neuroblastoma is the fourth most common type of cancer in children. According to the American Cancer Society, there are approximately 650 new cases of neuroblastoma each year in the United States. Despite significant educational efforts, improved diagnostic techniques, and rigorous therapies, neuroblastoma control remains static. To address this health issue, the objectives of this research was to investigate the use protein kinase C-iota (PKC- ι) inhibitors on the proliferation of neuroblastoma cells. Previous work has shown that inhibition of PKC- ι is a promising means by which to prevent and treat certain cancers. Here, we report the identification of a PKC- ι inhibitor (1H-imidazole-4-carboxamide, 5-amino-1-[2,3-dihydroxy-4-[(phosphonooxy) methyl] cyclopentyl]-,[1R-(1α, 2β, 3 β, 4 α)] (ICA-1) that targets a unique sequence (amino acid residues 469-475) in the catalytic domain of PKC-ι, inhibits PKC- ι activity and is effective in blocking the proliferation of BE(2)-C neuroblastoma cells. Our data support significant proof of concept that ICA-1 inhibits the proliferation of neuroblastoma cells and may be a novel chemotherapeutic for the treatment of patients with neuroblastoma.

Generation of Human Gene Knockout Cell Lines: Application to Assessment of Genotoxic Anticancer Drugs

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Background: DNA damage causes cancer, is used to treat cancer, and is responsible for many of the side effects of cancer therapies. It is thus important to understand how chemotherapeutic agents cause DNA damage and how cells cope with such damage, particularly in the context of human somatic cells. Many of chemotherapeutic agents currently used in the clinic target DNA topoisomerases; for example, camptothecin is a topoisomerase I inhibitor, while etoposide a topoisomerase II inhibitor. These agents, like ionizing radiation, have been shown to induce DNA double-strand breaks (DSBs), but clearly by distinct mechanisms. In human somatic cells, the roles of two major DSB repair pathways, homologous recombination (HR) and nonhomologous end-joining (NHEJ), in the repair of topoisomerase-induced DNA damage are not completely understood.
Methods: We have recently developed a system using the human pre-B cell line Nalm-6 that enables rapid production of knockout cell lines. Using this system, we generated human cell lines deficient for Rad54, a HR protein, and/or DNA ligase IV (Lig4), a critical NHEJ ligase. Cells lacking Artemis, a nuclease involved in NHEJ, were also created by gene targeting using Nalm-6. Sensitivity assays were performed by clonogenic survival assays or by using the CellTiter-Glo Luminescent Viability Assay kit (Promega).
Results: We find that NHEJ is critical for repairing topoisomerase II- as well as low-dose irradiation-induced DNA damage, while HR is important for repairing topoisomerase I-induced DNA damage. Intriguingly, NHEJ negatively affects survival of cells treated with the topoisomerase I poison camptothecin. We also find that loss of Artemis not only leads to increased camptothecin resistance, independently of Lig4, but also alleviates hypersensitivity of Lig4-null cells to topoisomerase II inhibitors.
Conclusions: 1) HR and NHEJ have different roles in the repair of topoisomerase-mediated DNA damage. 2) Our data have significant implications for chemotherapy involving topoisomerase inhibitors. 3) A series of human gene knockout cell lines are useful in assessing cellular DNA damage and repair induced by chemotherapeutic agents.

Magic Bullets against the Autoimmune Diseases

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Abstract

In 1908 Paul Ehrlich was awarded the Nobel Prize for discovering Salvarsan, a "magic bullet" that was more toxic for the spirochaete of syphilis than for man, and for showing that immune reactivity against host components did not normally occur but, as an abnormality, could be the basis of many disease processes. Today, knowledge of the autoimmune diseases has reached the stage of showing how they can be cured by "magic bullets" against the forbidden clones of B and T lymphocytes that cause them. The magic bullets can be made by isolating the target autoantigen and coupling it to a cytotoxic moiety, such as bungarotoxin or a molecule containing a radioactive element such as ^{131}Iodine emitting short-range radioactivity in the form of beta particles (electrons). Such therapeutic molecules, administered intravenously, would home cytotoxically on the lymphocytes of the pathogenic forbidden clones, selectively destroying them. The autoantigen for rheumatoid arthritis, calpastatin, is already available for use in this way, and so is that for the probably autoimmune lethal stage of HIV infection, AIDS, in the form of the CD4 structure on helper T lymphocytes. Additionally, dangerously severe autoimmune diseases can be cured by chemical or radiological generalised immune ablation, which will destroy the forbidden clones, followed by immune restitution with autologous stem cells, taken previously from the patient's own bone marrow. Because unlucky semi-random somatic mutations in immunoglobulin variable region (V) genes are responsible for occurrence of the pathogenic forbidden clones, they are unlikely to recur in the regenerated immune system (Burnet's Forbidden Clone Theory) and this has been demonstrated therapeutically in cases of severe scleroderma.

Nicotinamide Mechanisms of Neuroprotection in Stroke

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Background: Stroke is a major cause of death in the USA. Although stroke can be treated by removing intracerebral clots or by administration of clot dissolving agents, therapy for stroke is inadequate. Patients frequently do not recover normal speech or motion following strokes. The current work explores the development of a new therapeutic agent, nicotinamide, for recovery from stroke.
Methods: The focal ischemia and reperfusion model of stroke in rats was used, as well as the global ischemia and reperfusion model in mice. Mechanistic experiments were performed with intracerebroventricular t-butylhydroperoxide treated mice. Nicotinamide was adminstered at various doses and various times before or after ischemia and reperfusion. Brain cell damage was examined by light and electron microscopy. The brain levels of pyridine dinucleotides, glutathione and ATP were measured. DNA fragmentation and poly(ADP-ribose) polymerase activity were assessed.
Results: Mechanistic studies show that glutathione is oxidized, NAD and ATP levels decrease during oxidative stress in the brain. Poly(ADP-ribose) polymerase is activated and DNA fragmentation occurrs during oxidative stress. Ischemia and reperfusion result in a central necrotic core surrounded by a limit that includes apoptotic cells. Nicotinamide used as a pretreatment or post-treatment is able to prevent both necrosis and apoptosis in the brain suffering from ischemia and reperfusion. Nicotinamide is partially effective even when given 6 hours after reperfusion. Nicotinamide increases brain NAD and ATP levels and regulates poly(ADP-ribose) polymerase activity.
Conclusions: Nicotinamide is an effective and safe neuroprotective agent. The mechanism of nicotinamide neuroprotection involves inhibition of poly(ADP-ribose) polymerase and stimulation of NAD synthesis. Poly(ADP-ribose) polymerase regulates the activities of a number of enzymes and genes. These results will be discussed in terms of caspase activation and the induction of IAPs.

Author's disclosure statement: The author is part owner of the US patent for the use of nicotinamide as a neuroprotective agent in stroke and other conditions.

Prevention of Mucositis and Improvement in Compliance of Head & Neck Cancer Patients undergoing Radio-Chemotherapy by Curcumin

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Background: Oral mucositis (OM) is a common complication and a dose limiting toxicity in up to 90% of head & neck cancer patients (HNCP) undergoing radio-chemotherapy (RCT). Its adverse effects on schedule of therapy, quality of life and economic burden to the patients and system warrant an urgent need for a potent cell specific radioprotector. Several adjuvant agents like folic acid, Vit-E, antibiotic mouth rinse etc have been tried to prevent OM without remarkable success. Curcumin has shown radioprotective as well as radiosensitizing potential in *in vitro* studies with its antioxidant, free radical scavenging activity and ability to arrest cell cycle in G2 and M phase in malignant cells. Objective of this pilot study was to evaluate effects of curcumin on OM in HNCP under going RCT.
Methods: 95 HNCPs were given conventional RCT and 109 HNCP were given 2 gm of curcumin/day orally in 3 divided doses in addition to RCT for two months starting from 3 days before planned radiation. OM gradation as per WHO oral toxicity scale was done weekly for the whole RCT period and follow-up was continued for 6 months in both the groups. Incidence, onset and duration of OM in each grade, and patient compliance in term of uninterrupted completion of scheduled RCT were compared in both controlled and curcumin treated group by Chi-square test ($P \leq 0.05$).
Results: There was a significant decrease in incidence of grade III and IV OM ($P \leq 0.001$) among curcumin treated group. Patient compliance in terms of completion of scheduled RCT increased from 53% to 89.0% ($P \leq 0.001$).

Mucositis characteristics	Control (n = 95)	Trial (n = 109)
Total Patients with OM (%)	88 (92)	56 (51)
Gr-III and Gr-IV (%)	49 (51)	14 (13)
Mean onset of any OM (Gy)	19.1 (17.5-20.6)	26.9 (24.5-29.3)
Mean duration of any OM (days)	33.8 (28.9 - 38.7)	13.3 (11.2 - 15.3)
Duration of OM in Gr-III & IV(days)	39.7 (30.4-49.3)	17.1 (13-21.3)
Patients completing scheduled RCT (%)	50 (53)	97 (89)

Conclusion: 1. Curcumin showed a significant adjuvant protective activity against RCT induced OM in HNCP 2. Long term follow-up required for its effects on recurrence and survival in HNCP.

Preparation and Evaluation of Mucinated Sodium Alginate Microparticles for Oral Delivery of Insulin

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Background: Effective oral insulin delivery remains a challenge to the pharmaceutical industry. A novel formulation of insulin using new biomaterials is necessary to formulate and administer insulin via the oral route. Aims: 1) To develop new biocompatible delivery materials through cross-linking for the delivery of insulin. 2) To study the level of protection of insulin against degradation when formulated using the new biomaterial. 3) To study the *in vitro* and *in vivo* release of the insulin and its effect on blood glucose level in diabetic rabbits.
Methods: Cross-linking of mucin-sodium alginate of ratios 0:1 to 1:3 was effected in liquid paraffin using acetone at -30 ^{o}C. The microparticles were diffusion-filled and further coated with 1.5 % w/v cellulose acetate phthalate in acetone at -30 ^{o}C. Diabetes was induced in 32 rabbits weighing between 1.8-2.5 kg using 120 mg/kg of alloxan and these used for the anti-diabetic assessment. Insulin-loaded microparticles containing 50 IU/kg was given to the rabbits in 2 ml of distilled water using gastric intubation using distilled water as the negative control and 50 IU/kg subcutaneously as the positive control.
Results: The microparticles formed were generally multi-particulate, discrete and free flowing. Before insulin loading, microparticles were round and smooth, becoming fluffier, less spherical and larger with rough and pitted surface after insulin loading. The mean dissolution time of insulin from the microparticles prepared with sodium alginate, mucin, sodium alginate: mucin ratios of 1:1, 3:1 and 1:3 were 11.21±0.75, 3.3±0.42, 6.69±023, 8.52±0.95 and 3.48±0.65 (min) respectively. The percentage blood glucose reduction for the subcutaneously administered insulin was significantly ($p < 0.001$) higher than for the formulations. The blood glucose reduction effect produced by the orally administered insulin-loaded microparticles prepared with 3 parts of sodium alginate and 1 part of mucin after 5 h was, however, equal to that produced by the subcutaneously administered insulin solution.
Conclusion: This study shows that the oral route could be an effective alternative for the delivery of insulin using this polymer cross-link.

Trends in Co-Occuring Diseases in Patients Treated for Alcohol and Drug Problems in Canada.

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Background: Understanding the disease experience of alcohol and drug patients (AD) is needed for rational decision-making in health care and disease prevention. Aim: 1) To improve measurement of co-occurring disease (co-morbidity) in AD patients using population-based data 2) to easily identify reduced co-morbidity using a new more sensitive measure.
Method: Co-morbidity was measured using the standardized morbidity ratio (MR), comparing 1985-86 Hospital Medical Records Institute data for all inpatient cases(n=52,200) with primary (P) or secondary (S) alcohol or drug diagnoses in all Ontario hospitals to data for all patients in all Canadian hospitals, using indirect age-sex standardization and adjusting for multiple diagnoses. The modified MR (mMR) was used to improve measurement and visual display (graphing) of reduced co-morbidities.
Results: Compared to all patients, AD patients had more co-morbidity. Generally, MR was higher in patients with primary (P) rather than secondary (S) AD diagnoses Patients treated for the use or misuse of prescription drugs had the highest MR (MR_P=13.3 and MR_S=3.5). MR was intermediate for patients who used illegal drugs (MR_P=8.9 and MR_S=4.7), and lower for alcohol patients (MR_P=6.7 and MR_S =4.1). Co-morbidity in alcohol patients stemmed from practically all diagnostic categories, but co-morbidities in drug patients were due to fewer diagnostic categories. AD patients had particularly high MR for mental disorders, infections and parasitic conditions, and injuries and poisonings. Cocaine cases had high MR for infectious and parasitic diseases, and diseases of the skin and subcutaneous tissue. Amphetamine cases had high MR for diseases of the digestive system, and of the musculoskeletal system and connective tissue. Graphing results using the mMR clearly show AD patients had reduced co-morbidity from complications of pregnancy, childbirth and the puerperium, and from conditions originating in the perinatal period, but a higher rate of congenital anomalies.
Conclusion: 1) AD patients had more co-morbidity than all patients combined, with co-morbidity involving more diagnostic categories for alcohol than drug patients, but they had less co-morbidity related to reproduction than all patients. **2)** mMR is a sensitive measure of reduced co-morbidity.

TOPICAL IMMUNOTHERAPY WITH DIPHENYLCYCLOPOPENONE IN VITILIGO: A PRELIMINARY EXPERIENCE

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Background: Despite recent significant therapeutic advances, vitiligo remains as a clinical conundrum. Topical immunotherapy has been extensively tested in the treatment of various dermatologic disorders especially those believed to have an immunologic basis. The aim of this study was to evaluate the role of topical DPCP in the treatment of vitiligo.
Methods: Nineteen patients with limited vitiligo lesions were enrolled in this study. After sensitization with 2% DPCP, progressively higher concentrations beginning at 0.001% were applied weekly for 6 months to depigmented skin. The maximum concentration of DPCP in acetone was 2%.
Results: Thirteen of 19 patients were evaluated at the end of 6 months. Four patients with focal vitiligo, 3 patients with vitiligo vulgaris, and 3 patients with segmental vitiligo showed marked (grade 3) repigmentation.
Conclusion: Marginal and central repigmentation with hyperpigmented borders was seen in the majority of lesions. Further controlled trials should be undertaken to evaluate the use of topical DPCP in vitiligo, as this preliminary study is encouraging.

Studies on Interactions of Water Soluble Vitamins with Zinc Through cell, Protein and Animal Models in Health and Disease

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Background: Zinc (Zn) is required as a catalytic, structural and regulatory ion for enzymes, proteins and transcription factors and is thus a key trace element in many homeostatic mechanisms of the body. Vitamins like riboflavin, nicotinic acid, thiamine, folic acid and ascorbic acid have functional groups capable of forming complexes with Zn. However, the interaction of water-soluble vitamins with Zn has not received much attention.
Methods: We have examined the Zn-vitamin interactions at a variety of conditions like different Zn concentrations, different cell and protein models and under normal and oxidative stress (OS) conditions. The interactions were studied *in vitro*, by using erythrocytes under deficient, normal and excess Zn states.
Results: Under Zn-deficient state, thiamine significantly enhanced the erythrocyte Zn uptakes ($p<0.05$), whereas ascorbic acid and riboflavin inhibited it ($p<0.05$). In another study, an in vitro erythrocyte Zn uptake was compared among healthy and diabetic subjects and it was found that Zn uptakes of healthy subjects were 17-52% higher than those for diabetic subjects. Furthermore, erythrocyte super oxide dismutase, plasma ascorbic acid and status of riboflavin were negatively correlated with Zn uptakes in healthy subjects ($p<0.01$). These interactions were also studied in precision cut rat liver slices, where it was found that folic acid showed inhibitory effect on Zn uptake under both normal and OS conditions as seen by dose response curves. Ascorbic acid showed marked enhancing effect on Zn uptake under OS. These *in vitro* interactions were confirmed in vivo using male Wistar rats. The 21 days old rats were used to examine the effect of niacin supplementation on Zn absorption under chronic OS generated by tert-butyl hydro peroxide at a dose of 0.2 mM/Kg body weight. Niacin supplementation increased the Zn absorption and improved antioxidant enzyme profile. The albumin being the major Zn carrier protein in plasma and the albumin bound Zn (ABZn) comprises 80% plasma Zn. Folic acid and thiamine significantly enhanced the ABZn ($p<0.010$), while nicotinic acid inhibited Zn binding to albumin.
Conclusions: These results collectively suggest that vitamins are playing an important role in distribution of circulating Zn among albumin, blood cells and liver and giving a new dimension to their functionality in Zn metabolism in health and disease conditions.

Association of Fluoroquinolone and ESBL-Resistance in Gram-Negative Organisms from Oncology Patients of Lagos University Teaching Hospital (LUTH), Nigeria

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Background: This study determined the gram-negative bacilli associated with various cancer infections and defined fluoroquinolone susceptibility and Extended-Spectrum Beta-Lactamase production in isolated strains.
Methods: Materials for research were blood culture, urine, aspirates, fluids and swabs from cancer wounds. Samples were cultured and organisms isolated were determined using API system (Bio-Merieux). Antimicrobial resistance was estimated by the disc diffusion method according to NCCLS/CLSI recommendations and ESBL detection was carried out using the Double Disk Synergy Test method
Result: Of the 103 strains isolated 22 (21.4%) were found to be resistant to only ciprofloxacin. Only 1 of these resistances to ciprofloxacin was observed to have an accompanying production of ESBL. Of the 7 isolates that had resistance to a combination of two fluoroquinolones, 2 (28.6%) were found to be ESBL-producers. Cross resistance to the 3 quinolones tested, occurred in 40 (38.8%) of the strains isolated. The strains in this group were observed to be associated in most of the cases with MDR [35 (37.5%)] and production of ESBL [16(41%)]. This group was observed to be predominant amongst strains of *E.coli*, *Pseudomonas* spp and *Klebsiella* spp.
Conclusion: Cross-resistance to fluoroquinolones has emerged amongst these clinical isolates and more worrisome is its association with ESBL-Production and Multidrug Resistance. Multi-drug resistance may be one of the contributing factors to the high mortality rate amongst these group of patients reported in developing countries. Antibiotic resistance surveillance is thus of utmost importance in contributing to the reduction of the high morbidity and mortality rate reported amongst cancer patients in this part of the world.

Clinical Applications of Ghrelin

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Background: Ghrelin is a 28-amino-acid peptide hormone that was discovered in 1999 as an endogenous ligand for the growth hormone (GH)-secretagogue receptor (GHS-R). Ghrelin plays a critical role in a variety of physiological processes including stimulation of GH secretion and food. These actions of ghrelin should be invaluable for the development of novel treatments and disease diagnostic techniques.
Methods: Study 1: we attempted to evaluate the clinical response to repeated ghrelin administration in patients with anorexia caused by functional disorders. Subjects were included in this study, who 1) are diagnosed with functional anorexia, 2) are lean, and 3) exhibit decreased FI. Subjects received an intravenous infusion of 3 microg/kg ghrelin for thirty minutes twice a day for two weeks. We investigated the effects of ghrelin administration on food intake (FI), appetite, hormones, and metabolic parameters. Study 2: we evaluated the effects of ghrelin administration on physical performance and body composition in patients undergoing elective total hip replacement (THR) as treatment for osteoarthritis (OA). Thirty-two patients were assigned to two groups of sixteen subjects each; the ghrelin group received intravenous injections of 2 microg/kg ghrelin, while the placebo group received vehicle alone. Subjects received twice daily injections for three weeks beginning one week before surgery.
Results: Study 1: Ghrelin administration tended to increase daily FI in comparison to levels before and after completion of treatment, although this difference that was the primary endpoint of this study did not reach statistical significance. Hunger sensation was significantly elevated at the end of drip infusion. Study 2: While ghrelin significantly increased lean body mass after the three-week injection period, it did not affect muscle strength or walking ability. Significant decreases in fat mass and GH responses to ghrelin injection were also observed.
Conclusions: Ghrelin treatment has stimulatory effects on appetite in patients with functional anorexia. On the other hand, ghrelin administration did not provide any favorable effect on physical performance in patients with OA undergoing THR, despite increased lean tissue reserves. Further studies remain necessary to confirm the efficacy of ghrelin treatment for anorexia- and catabolism/aging-related disorders.

Effect of Diabetes Mellitus on Pharmacokinetics (PK) and Pharmacodynamics (PD) of Immunosuppressive agents: Ciclosporin, Tacrolimus and Mycophenolic Acid

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Background: A large percentage of kidney transplant recipients are diabetics but little is known on the effect of diabetes on the disposition or effect of immunosuppressive agents.
Methods: The PK and PD studies included 42 and 32 stable kidney transplants, sampled for 12 or 2 hours post dose, respectively. Approximately 50% of patients were diabetic (D) that were demographically matched with non-diabetic (ND) controls. Immunosuppressive agents' total, unbound and metabolite concentrations were measured by LC-MS/MS. Markers of T-cell activity (ATP concentration in CD4 cells, intracellular cytokines IL-2, IFN-□ and TNF-□ in CD3 cells) and B-cell activity (co-stimulatory proteins CD54 [ICAM-1], CD86 [B7.2] and CD95 [Fas antigen] in stimulated CD19) were determined. Inosine monophosphate dehydrogenase (IMPDH) activity was also measured in some patients.
Results: *Pharmacokinetics:* As summarized in the Table, both ciclosporin (CsA) C_{max} and AUC was reduced in Ds and CsA free fraction was higher. Tacrolimus absorption was delayed with no effect on the overall AUC. Diabetes delayed mycophenolate mofetil (MMF) absorption rate but not the enteric coated Na-MPA. The concentration of some metabolites of ciclosporin or MPA was lower in Ds.
Pharmacodynamics: The mean ± SD of ATP concentration was 298 ± 78 ng/mL in D and 359 ± 86 in ND patients (*P=0.021*). Expression of intracellular IL-2 in CD3 cells was lower in D (mean florescence intensity: D 104 ± 42, ND 145 ± 74; *P=0.03*). Also CD95 cell surface expression on B-cells was lower in Ds (D: 73 ± 28 ND: 114 ± 68; *P=0.01*) however despite a trend, expression of CD54 or CD86 did not differ. IMPDH activity was 17.5 ± 2.8 vs. 46.6 ± 2.5 nmol XMP/h/μgprotein, in D and ND, respectively (P<0.0001).
Conclusions: Diabetes variably affect the PK/PD of immunosuppressive agents. Adjusting immunosuppressive dose guided by the PD markers may prove useful in dose individualization of immunosuppressive agents in diabetics.

	Absorption rate	Exposure (AUC)	Free fraction (fu)	Metabolites
Cyclosporine	Delayed	?	Increased	? AM1, ? AM19
Tacrolimus	Delayed	?	??	? Clearance, metabolites??
Mycophenolic Acid	Delayed as MMF ? EC-Na-MPA	?	?	? MPAG ? Acyl-MPAG

Chemotherapy of advanced colorectal carcinoma under hemodialysis

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Background: Recent progress of hemodialysis therapy (HD) has prolonged the life span of patients sufferring from end stage renal disease (ESRD) and it increased the complication of malignant tumors. It is reported that the prevalence of colorectal cancer in HD patients is higher than that in a control population. Saltz regimen; combination therapy of irinotecan, 5-fluorouracil, and leucovorin is widely used for the treatment of metastatic colorectal cancer after the large randomized phase III trials. Chemotherapy of HD patients is difficult because there are few pharmacokinetic data of most cytotoxic agents much less combination chemotherapy. We encountered a patient of colorectal carcinoma on HD. It was decided to give combination chemotherapy with monitoring the pharmacokinetics of the patient.
Methods: CPT-11 was administered just after HD at a dose of 50 mg/m^2 by 90-min intravenous infusion; followed by *l*-LV 10 mg/m^2, administered over the course of 15min; and 5-FU (400 mg/m^2), given by bolus intravenous injection after *l*-LV. Three drugs were given on days 1, 8 and 15 of a 35-day cycle. Blood samples were collected before administration and at 0.5, 4, 12, 24 hours after administration. Plasma concentration of CPT-11, SN-38 and 5-FU were compared to those in reference control values without ESRD. The plasma concentration of CPT-11, SN-38 and 5-FU were analyzed using high performance-liquid chromatography (HPLC).
Results: The pharmacokinetic results for each post administration value were not statistically different compared with the data of normal renal function. As for side effects, he experienced grade III hematological toxicity, which was easily controlled with G-CSF.

Time after administration (hrs)	0	0.5	4	12	24
CPT-11 □ng/ml□	0	820-845	610-790	340	95
SN-38 □ng/ml□	0	8.4-12.0	7.5-8.2	5.2	4.4
5-FU □ng/ml□	0	7,200-12,300	0	0	0

Conclusions: These data suggest that Saltz regimen can be feasible for colorectal cancer patients receiving HD without dose reducing

In vitro Activities of Extracts from Naturals Plants on the Human Filaria *Loa loa*

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Background: *Loa loa* is a human filaria endemic to West Africa. Current drugs such as Diethylcarbamazine (DEC) and Ivermectin induce severe adverse reactions like encephalitis and patient death in high microfilaremic individuals. Therefore there is a need for new drugs. Plants are known as potential nematocids, but no study has been carried out to examine their effect on *Loa loa*. Aim: to screen nematocidal plants used in traditional medicine for their filaricidal activity.
Methods: Plants were collected according to their traditional usage an identified using botanic criteria. Extracts were obtained by using different organic solvents (Dichloromethane, Methanol, Water). The resulting products were dissolved at 1mg/ml in Dimethylsulfoxid (DMSO). Co cultures of extract and filaria were made in duplicate using a 96 well cell culture plaque containing LLCMK2 cells, MEM medium with 10% Fetal calf serum and antibiotics. 5 *Loa loa* microfilaria were added in each well and plaques were incubated at 37°C in 5% CO_2 atmosphere for 120 hours. The effects of plant extracts were measured by observing the motility and mobility of filaria in wells containing different concentrations of extract compared to negative controls (no extract or drug in the well) and positive controls (DEC or Ivermectin in the well) at different concentrations.
Results: Extracts from seven plants species of the *Euphorbiaceae*, *Caesalpiniacae*, *Compositeae*, *Mimosaceae*, *Ochnaceae*, *Rutaceae*, *Annonaceae* family had microfilaricidal activity at concentrations of 95.6µg and 47.8µg, while plants from *Lecytidaceae* had no effects on filaria at these concentrations.
Conclusion: Crude extracts of some plants used in traditional medicine have *in vitro* effect on the human filaria *Loa loa*.

The Role of Ancient Iraqi People (Sumerian, Assyrian, Babylonian and Arabian) in the Development of Medicine as Viewed by Western Writers

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Background: The region of Iraq was historically known as Mesopotamia and was home to the world's first known civilizations These civilizations produced some of the earliest writing and some of the first sciences and medicine of the world; hence its common epithet, the "Cradle of Civilization". The aim of this paper is to study the views of the distinguished western writers regarding the role of ancient people of Iraq in the development of Medicine.
Methods: the views of the distinguished western writers regarding the role of ancient people of Iraq in the development of Medicine were reviewed and studied.
Results: Samuel Noah Kramer considered the considered the Sumerian pharmacological tablets as the first pharmacopoeia. In summarizing the effects of Arabian in the development of medicine Stubb and Bleight stated in 1931"With Arabs began the real craft of apothecaries". Meyerhof in 1944 wrote in his book "Pharmacology during the golden age of Arabian Medicine" "The establishment of hospitals was originated by Arabians"
Conclusions: The ancient Iraqi people played an important role in the early development of medicine.

Retargeting anticancer drugs to drug resistant cancers by using polymer Biotransport technology. Clinical proof of the concept

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Background: Anthracyclines are amongst the most widely used anticancer agents in man, but are limited by toxicity considerations, as well as by inherent or induced drug resistance. We have recently discovered that nonionic block copolymeric surfactants, i.e. hydrophobic polyethylene oxide polypropylene oxide block copolymers (Pluronics) can considerably reduce drug resistance of various tumour cells to anthracyclines and other cytotoxic drugs. Following this finding, we have developed a Pluronic-based formulation of doxorubicin (SP1049C) that is thermodynamically stable, safe and provides doxorubicin with high efficacy against both drug resistant and drug sensitive tumours.
Methods: Safety and plasma pharmacokinetics of SP1049C were evaluated in phase I clinical trial that was curried out in 26 advanced cancer patients; and efficacy of the product was tested in phase II clinical trial, in 21 patients with metastatic adenocarcinoma of the esophagus.
Results: The results of phase I trial demonstrated comparable safety and PK profiles of SP1049C to that of doxorubicin. The phase II trial revealed that the product has a high anticancer activity. The overall responce rate to SP1049C was 47%, (95% CI: 24 -71), and medial survival was 10 months, which compares favourably with other most active single agents tasted in this indication.
Conclusions: The performed clinical studies have assuared a further clinical development of SP1049C. At present it is entering an expanded international clinical phase II/III efficacy program including a single 300 patient pivotal phase III trial in patients with upper gastrointestinal tract.

Novel reporter probes for HSV1-*tk* gene expression

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Herpes simplex virus type-1 thymidine kinase (HSV1-*tk*) gene is being used as a suicide gene for gene therapy of cancer. Cancer cells are transduced with a retroviral vector carrying HSV1-*tk* gene. The gene expressed in the proliferating cells produces the HSV1-Tk enzyme that selectively phosphorylates a guanosine derivative, ganciclovir, to its monophosphate. The ganciclovir monophosphate is then converted to its di-phosphate form by the cellular kinases and/or HSV1-TK, and finally to the triphosphate form by cellular kinases. The ganciclovir tri-phosphate becomes a pro-drug and inhibits DNA polymerase, thus kills the tumor cells.
Malignant tumors have been successfully treated in animal models with suicide gene therapy using HSV1-*tk* gene and ganciclovir. However, clinical results with this method showed that gene delivery to the tumor cell in human was not sufficient. Therefore, an *in vivo* method to assess the HSV1-TK enzyme activity after gene transfer is required for the optimization of gene delivery and establishment of treatment efficacy. Positron emission tomography (PET) is a non-invasive modality for *in vivo* imaging of HSV1-*tk* gene expression using reporter gene and reporter probe, and can provide repeated and quantitative assessment of the expression of genes in tissues and organs.
Non-invasive imaging of transgene expression involves the appropriate combination of a reporter gene and a reporter probe. Model systems have been established and validated by HSV1-*tk* gene as a reporter gene, and radiolabeled pyrimidine nucleoside and acycloguanosine analogues as reporter probes. Reporter probes can be used (i) to image vector targeting and level of HSV1-*tk* expression, (ii) to image the regulation of endogenous genes and signal-transduction pathways; and (iii) to monitor and quantitatively assess the expression of a second transgene that is cis-linked to the reporter gene by an internal ribosome entry site sequence.
Various radiolableld reporter probes have been developed during the past decade, and these are primarily purine and pyrimidine nucleoside analogues, such as ^{18}F-FHPG, ^{18}F-FHBG, ^{18}F-FMAU, ^{18}F-FIAU, ^{18}F-FEAU, ^{18}F-FFAU etc., and also radioiodinated analogues of FIAU. Among these probes, ^{18}F-FHBG, ^{18}F-FIAU and ^{18}F-FEAU have been studies extensively. ^{18}F-FHBG and ^{18}F-FEAU have been recognized as ideal probes for PET imaging of HSV1-*tk* gene expression. We have studies these probes in the animal models and demonstrated that both ^{18}F-FHBG and ^{18}F-FEAU have efficacy for imaging of HSV1-*tk* gene expression, and they can be used selectively in PET imaging of native and mutated HSV1-tk gene expression. Thus ^{18}F-FEAU is suitable for PET imaging of the native HSV1-*tk* gene expression; and ^{18}F-FHBG is suitable for PET imaging of mutated HSV1-*tk* gene expression. This presentation will focus on these novel probes used for PET imaging of HSV1-*tk* gene expression with an emphasis on their application in pre-clinical animal models and human studies.

Drug-Drug and Drug-Biomolecule Photoinduced Reactions in Preparations for Topical Use

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Background: There is a growing concern for the adverse effects caused by exposure to indoor/outdoor light after assumption of drugs. The first concern is obviously for topical preparations. The rather sparse literature on phototoxicity rarely examines the chemical reactions underlying to the biological effect. In general, there is a feeling that oxygen activation is the main mechanism, but the example below show that this is not always the case.
Methods: *In vitro* studies were carried out by irradiating aqueous solution of the drugs under indoor/outdoor light at a measured flux. The photoproducts were isolated and the structure determined. The exploration was repeated in the presence of heterocycles mimicking the behaviour of nucleotides. Time-resolved studies for the detection of chemical intermediates were also performed. These studies and the structure of the products allowed to propose viable mechanisms, here concerning some UV filters used in sunscreens as well as some bactericides with eye-toxicity.
Results: Dibenzoylmethanes (DBM, in the tautomeric form under the applicative conditions) are a virtually constant component of commercial sunscreens in view of the high absorption in the UV-A. These products are quite photostable but are often associated with cynnamates in the preparations, because the latters absorb the UV-B. However, if the combination of the two filters is exposed to light, both are rapidly consumed via a photocycloaddition reaction. Furthermore, irradiated DMBs photo-add to a variety of molecules present in the skin, e.g. to fats.
As for antibacterials we refer here to fluoroquinolones used in eye drops, such as lomefloxacin (LOM), and to oxazolidinones such as linezolid, known to cause damage to the eye. In both cases a fast photoinduced decomposition takes place, with liberation of fluoride and formation of an aggressive intermediate (an aryl cation) that adds to a various compounds, including electron-rich heterocycles considered as a model of nucleotides, but not to water.
This negative effect support that exposure to light should be minimized (e.g. LOM has been found to be genotoxic), but also suggest that fluorinated heterocycles may be considered for a novel type of photodynamic therapy based on the selective addition to biomolecules by photoproduced aryl cations, veritable magic bullets.

Protective Effect of Dimebon on the TNF-alpha–Induced Lipid Disorders in Mice Brain

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Background: Dimebon (Dimebolin) is an antihistamine drug which has been used clinically in Russia since 1983. Recently Dimebolin has attracted renewed interest after being shown to have positive effects on persons suffering from Alzheimer's disease. Animal studies have shown that dimebon operates through multiple mechanisms of action, both blocking the action of neurotoxic beta-amyloid proteins and inhibiting L-type calcium channels, modulating the action of AMPA and NMDA glutamate receptors, and may exert a neuroprotective effect by blocking cytotoxic signals induced by proinflammatory citokines such as TNF-alpha,which are believed to play a central role in Alzheimer's disease. Inflammatory response induced by TNF-alpha suggests that this cytokine affects the phospholipid metabolism and subsequent production of eicosanoids, ceramide, and ROS that may potentiate brain injury.
Methods: This study included 65 male mice (weight: 20 ± 2 g, aerage ± SD). TNF-alpha (10mkg per mouse), dimebon (0,2 mg/kg) and their combination were injected to mice interperitonealy. Changes in level of phospholipids molecular species (phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine, sphingomyelin) and galactosylceramide in hippocampus, cerebellum and cerebral cortex within 30 min, 2, 4 and 24 hours after injection were detected by chromato-mass-spectrometry.
Results: Maximal changes in phospholipids and galactosylceramides contents of different molecular species after single TNF-alpha administration were found in the hippocampus, and were less expressed in the cerebral cortex and cerebellum after 24 hours. Dimebon itself did not induce changes in lipids spectrum in brain sections, but protected lipids against disorders induced by TNF-alpha in mice brain.
Conclusions: Modern strategies in the search of new therapeutic approaches are based on the multitarget properties of new drugs. According to our results TNF-alpha may serve as a new target for dimebon. Dimebon preventing lipids disorders in brain induced by TNF-alpha might have a positive anti-inflammatory effects, preventing the negative response of nerve cells to the pathological process.

The Role of cell membrane lipid environment in antigenic peptide structure-function

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Background : The cell membranes control the extra- and intracellular exchange. The lipid structure, their organization in the membrane and around membrane proteins but also their dynamics in the two dimensions of the membrane are all essential characteristics of each cell type, for their selective role in the cell traffic. The aim of our study was to address the role of the lipids on an HIV-1 vaccine epitope structure and on the interaction with target cell membranes : either CD4+ T cells or epithelial cells.
Methods: Biophysical, biochemical and biological methods have been used. Peptides derived from HIV-1 glycoprotein envelope gp41 were chemically synthesized or produced as recombinant peptides. To study lipid dynamics and domain organization due to protein interaction, liposomes of different lipid composition, mimicking either the viral membrane or target cell membranes were chemically obtained and fluorescence resonance energy transfer (FRET) has been used. Nuclear Magnetic Resonance (NMR) allowed defining peptide solution structure in presence of micelles. Binding of known HIV-1 antibodies to the epitopes inserted into liposomes were measured by enzyme linked immunoassays (ELISA).
Results: Different peptides from conserved regions of the HIV-1 envelope glycoprotein gp41 have been studied, including: 1) P1, the minimal Membrane Proximal Region (MPR) that permits interaction with mucosal galactosyl ceramide HIV-receptor and contains epitopes recognized by gp41-specific, broadly neutralizing IgGs, 2F5 and 4E10, and 2) P5, P1 extended at its N-terminal by a calcium binding site. The alpha-helical structure of the peptides on DPC micelles appeared pH-dependent. P1 was derivatized with phosphatidylethanolamine (PE) at its C-terminal and inserted into liposomes of varied lipid composition, thereby enabling P1 to move laterally within liposome bilayer. The Kds of both 2F5 and 4E10 IgGs measured on viral liposome and infectious virus are similar in the nM range and much lower than for the binding of the free P1 peptide. For P5, the role of cell calcium on its structure and its antiviral properties when in interaction with lipids was shown essential.
Conclusions/Significance: Thus, the defined lipid environment of MPER-derived peptides and environmental conditions (pH, calcium, ...) appear as essential for their structure and therefore for the design of an immunogen inducing broadly neutralizing antibodies to HIV-1, and also microbicides.

Protective effects of *Nigella sativa* extract and its components against chromium VI-induced toxicity in Nile tilapia (*Oreochromis niloticus*) and zebrafish (*Danio rerio*)

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Background: Chromium is an increasing health concern for aquatic environments, however, the mechanism of chromium toxicity in aquatic species is yet unknown. Hexavalent chromium VI (CrVI) is the dominant toxicant at some Superfund sites within Egypt.
Methods: The aim of the current study was to evaluate the genotoxicty potential of chromium VI (CrVI) in Nile tilapia (*Oreochromis niloticus*) and zebrafish (*Danio rerio*) using semi-quantitative reverse-transcription polymerase chain reaction (RT-PCR), and possible protective effects of *N. sativa*. The test fish within the two species were divided into eight groups and treated with Cr(VI) alone or in combination with the crude extract of *N. sativa*, *N. sativa* oil, or its derivative thymoquinone for 30 days.
Results: The semi-quantitative RT-PCR results indicated that treatment with Cr(VI) at 4.37 and 1.75 mg/l for tilapia and zebrafish, respectively resulted in a significant increase in hepatic and brain mRNA level of cytochrome 450 gene family including CYP1A2, CYP3A and CYP2E1 in both fish species compared to control group. Moreover, Cr(VI) was found to induce severe histological changes in liver, brain and gills of the tested fish. On the other hand, the combined treatment showed that mRNA level of CYP1A2, CYP3A and CYP2E1 decreased significantly in the groups treated with Cr(VI) plus *N. sativa* oil or thymoquinone compared to the groups treated with the crude extract or Cr(VI) alone accompanied with a significant improvement in the histological picture of the liver, brain and gills. However, N. sativa was found to be more effective.
Conclusion: It could be concluded that N. sativa is a promise candidate against DNA damage resulted from the exposure to different environmental pollutants.

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All abstracts are listed in alphabetical order of the presenting author.

Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*

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Abstract

Silver nanoparticles have been known to have inhibitory and bactericidal effects. Resistance to antimicrobial agents by pathogenic bacteria has emerged in recent years and is a major health problem. The combination effects of silver nanoparticles with the antibacterial activity of antibiotics have not been studied. Here, we report on the synthesis of metallic nanoparticles of silver using a reduction of aqueous Ag+ ion with the culture supernatants of *Klebsiella pneumonia*. Also in this investigation, these nanoparticles were evaluated for their part in increasing the antimicrobial activities of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*. The antibacterial activities of penicillin G, amoxicillin, erythromycin, clindamycin and vancomycin were increased in the presence of silver nanoparticles against both test strains. The highest enhancing effects were observed for vancomycin, amoxicillin and penicillin G against *Staphylococcus aureus*.

Effect of gentamicin on serum digoxin level in patients with congestive heart failure.

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Background: Gentamicin is frequently used to treat infectious diseases in patients receiving digitalis therapy. The aim of this study is to evaluate the effect of gentamicin on serum digoxin level.
Methods: Twenty-four diabetic patients and patients with congestive heart failure and twelve normal healthy volunteers were enrolled in this study. The patients received digoxin treatment 0.25 mg/day. Gentamicin in a dose of 80 mg i.m. twice a day for 7 days was prescribed for these patients to treat chest infection. Serum digoxin and creatinine levels were determined before and after gentamicin administration.
Results: Gentamicin induced a significant increase in serum digoxin level of diabetic patients and patients with congestive heart failure. Serum creatinine level increased significantly before and after i.m. injection of gentamicin.
Conclusions: The present study indicated that increase serum digoxin level when combined with gentamicin should be considered a risk factor for digitalis toxicity.

Controlling of Systemic Absorption of Gliclazide through Incorporation into Alginate Beads.

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Background: Gliclazide is an oral hypoglycaemic second generation sulfonyl urea drug which is useful for a long term treatment of non insulin dependent diabetes mellitus. However, the absorption rate of gliclazide from gastrointestinal tract is slow and varied among the subjects. Several studies on healthy volunteers and diabetic patients revealed that the time to reach plasma concentration (t_{max}) ranged from 2 hours to 8 hours following a single oral administration of 80 mg of gliclazide tablet. Aims: (1) to develop controlled release formulations of gliclazide using alginate beads. (2) to study the effect of processing conditions on the physical characteristics of the prepared beads and the in vitro release rate of the drug (3) to investigate the hypoglycaemic effect of the prepared gliclazide loaded alginate beads on the diabetic rabbits and to compare the developed systems with marketed conventional gliclazide tablets.
Methods: Gliclazide loaded Ca-alginate beads were prepared using ionotropic gelation method. The variation in polymers concentrations, stirring speed, internal phase volume and the type of surfactants in external phase were examined systemically for their effects on particle size, incorporation efficiency, flow properties of the beads and in vitro drug release rates. The in vivo studies involved measuring blood glucose level of 18 diabetic male New Zealand white rabbits treated (6 rabbits per treatment) with the following formulations (1) normal saline solution (2) marketed conventional gliclazide tablet (Gliclazide ®) (3) gliclazide loaded alginate beads.
Results: The average mean diameters of gliclazide beads decreased with decreasing polymer concentration, increasing speed of stirring, and increasing the internal phase volume. All prepared beads possessed excellent flowability. The swelling behaviour was strongly dependent on polymer concentration in the formulation and the pH of the medium. The in vitro release experiments revealed that the swelling is the main parameter controlling the release rate of gliclazide from the beads. In vivo studies on diabetic rabbits showed that the hypoglycaemic effect induced by gliclazide loaded alginate beads was significantly greater and more prolonged than that induced by the marketed conventional gliclazide tablet (gliclazide ®).
Conclusions: Alginate beads can control, improve and prolong the systemic absorption of the gliclazide through their mucoadhesive properties. This effect results in maintaining tight blood glucose level and improved patient compliance.

Pharmaceutical Analysis using Sequential Injection Analysis (SIA): A review of present Applications and future possibilities

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Background: The need for automation in pharmaceutical analysis has lead to the development of rapid and sensitive detection methods. Our aims are to develop robust and simple sequential injection analysis (SI) methods for the essay of pharmaceutical samples and to investigate various luminescence detection modes in combination with SIA system. The approaches compared will be spectrophotomety, micellar enhanced fluorescence, and lanthanide enhanced luminescence and chemiluminescence (CL).
Method: The development of analytical protocols for monitoring of various drugs is presented. The chemical system is developed based on the structural properties of the given drug. When compounds lack efficient chromophores the use of a metal ion or organic derivatizing agents that can form highly absorbing or fluorescent products is necessary. On the other hand lanthanides such as Eu(III), and Tb(III) are used to sensitize the luminescence of drug samples offering excellent analytical characteristics.
CL is also emerging as an efficient tool when coupled to flow techniques. Ru(III) complexes were used to develop simple and robust assay for group of common drugs such as chlorpheniramine (CPA) and ephedrine(EP) and some fluoroquinolone antibiotics. Several types of SI designs were employed.
The development of the analytical system thereafter hinges on the selection of the most suitable environmental factors that result in an enhanced signal-to-noise ratio. Therefore a systematic optimization protocol must be used for this purpose. Then the methods developed are validated and compared to standard and official methods.
Results: using spectrophotometric techniques penicillamine (PA) was complexed with Fe(II) ions in acidic media forming blue complex that absorbs strongly at 600 nm. A linear dynamic range for the determination of PA of 25-300 ppm was obtained with sampling frequency of 50 h^{-1}. PA and ephedrine were determined using tris(bipyridyl)ruthenium(II) as a CL reagent and potassium peroxydisulfate as an oxidant in the presence of light.. Derivatization of PA and EP with aldehydes produced a significant enhancement of the CL emission, leading to detection limits (LOD) of 0.1ppm for PA and 0.03 ppm for EP.
Optimum conditions for the determination of BRZ in pharmaceutical formulation were 0.6 % tergitol surfactant in the presence of 0.1 M lactose.
Piroxicam (PX) and ibuprofen (IB) were assayed using lanthanide sensitized luminescence. Eu(III) when complexed to PX resulted in a huge enhancement in the emission of the EU(III) bands.. which allow the determination of 100–1000 ppb of PX with LOD of 29 ppb. Recoveries of PX in pharmaceutical formulations and in urine samples were 100.87±1.7% and 97.57±2.0%. IB was determined after complexation with Tb (III) ions giving a detection limit of 1.0×10^{-7} mol/L.
Fluoroquoinolone antibiotics eg levefloxacin (LV), were determined using Ce(IV) ions as oxidant and tris(1,10-phenanthroline)ruthenium(III) as the CL reagent giving characteristic orange emission with LOD of 0.02 ppm.
Conclusions: SIA is a powerful tool when coupled to sensitive luminescence methods for the determination of drugs in the pharmaceutical formulations and in biological fluid. This combination results in an increased sampling frequency and an enhanced sensitivity. The use of micelles has lead to an increase in the solubilisation of BRZ and an improvement in the sensitivity of the method. Lanthanide sensitized luminescence lead to a sensitize detection of the drugs with emission in the longer wavelength of the lanthanide ion that achieve a better selectivity of the methods. Future work include the development of methodology for the determination of drugs in various environmental samples such as round water and sewage, in order to cope with the increase problem of pollution brought about by the continuous charging of drugs into the environment.

Clinical Pharmacokinetics of Gentamicin: Estimation of Initial Dosing Parameters in Hospitalized Patients at Kuwaiti Hospital.

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Background: Gentamicin has a narrow range between its therapeutic and toxic blood levels. This has prompted the development and wide use of pharmacokinetic dosing equations in order to maximize drug safety and efficacy. Dosing equations commonly rely on estimating gentamicin clearance (Cl_{gent}) and volume of distribution (V_d). These parameters are subject to considerable variability's. The objective of this study was to: (a) develop equations for estimating Cl_{gent} and Vd based on Kuwaiti population and (b) evaluate these equations by comparison with other methods in their predictive ability to estimate Cl_{gent} and V_d.
Methods: Cl_{gent} and V_d were calculated in 47 patients (group 1) using the Sawchuk-Zaske method. Regression analysis was used to derive a correlation between creatinine clearance (Cl_{cr}) and Cl_{gent}, V_d and actual body weight (ABW). Based on actual Cl_{gent} and V_d values, the predictive ability of the estimated parameters from the regression equations was validated and compared with 4 methods, using mean error (ME) (bias), mean squared error and root mean squared error (MSE and RMSE, respectively) (precision). All equations were also evaluated in an independent second group (group 2) of 23 patients.
Results: (a) The mean (±SD) for Cl_{gent} and V_d, was 4.0 (±1.8) $L.h^{-1}$ and 16.8 (±6.7) L, respectively. (b) The derived equations were: Cl_{gent} = (0.760) (Cl_{cr})+1.117 (r = 0.701) and V_d = (0.165)(ABW)+5.604 (r = 0.532). In comparison to the four published methods, the derived equations were found to be less biased (ME=0.00), and more precise (MSE=1.68, RMSE=1.02) in predicting Clgent ($p<0.05$), and less biased (ME=-0.01) with no difference in precision (MSE=36.22, RMSE=4.59) in predicting Vd, ($p>0.05$). This precision was confirmed in the second group of 23 patients, where the derived equations were less biased (ME=-0.1) and more precise (MSE=3.22, RMSE=1.48) in predicting Cl_{gent} ($p<0.05$) and no differences were found for prediction of V_d ($p>0.05$).
Conclusion: The equations developed in this study provided a reliable estimation of Cl_{gent} and V_d. It is planned to use them at Kuwait Hospitals help provide more individualized patient dosing information to physicians.

Complexation of Itraconazole with Cyclodextrins for Enhanced Solubility, Dissolution and Bioavailability

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Background: Itraconazole is an antifungal agent whose poor aqueous solubility restricts its use for the treatment of oropharyngeal candidiasis, which is the first symptom of HIV infection. Therefore, the aim of this study was to prepare itraconazole-cyclodextrin inclusion complexes in solid state by different methods in order to enhance the solubility, dissolution and bioavailability of itraconazole.
Methods: The formation of inclusion complexes between itraconazole and α-, β-, γ-, and hydroxylpropyl-β-cyclodextrin (HP-β-CD) were assessed using phase solubility techniques. Solid state inclusion complexes between itraconazole and cyclodextrins were prepared using supercritical (SC) CO_2 and conventional methods. The physico-chemical properties of the products were characterized by UV, DSC, FTIR, PXRD and SEM. Dissolution amounts of the products obtained by different methods were measured in gastric fluid. Finally, pharmacokinetic studies of the inclusion complexes were conducted in blood, liver and kidney of male Wistar rats to assess the bioavailability of the prepared complexes.
Results: The aqueous solubility of itraconazole increased linearly as a function of cyclodextrin concentration according to the rank order: HP-β-CD > β-CD > γ-CD > α-CD. Inclusion formation was influenced by the preparation technique. Products obtained by the SC CO_2 method were among the ones showing the highest interaction between itraconazole and the CDs, leading to about three times higher dissolution amounts than pure itraconazole. Temperature, pressure and drug:CD ratio had significant effects on the inclusion yield. *In vivo* drug pharmacokinetic studies showed that the itraconazole-β-CD product prepared using SC CO_2 results in higher bioavailability of itraconazole than those obtained by physical mixing or coprecipitation methods.
Conclusions: Cyclodextrins significantly improved the solubility of itraconazole in aqueous solutions, which should improve the therapeutic effects of itraconazole against antifungal infections. SC CO_2 method proved to be an effective technique for preparing solid complexes between cyclodextrins and itraconazole. Since SC CO_2 method has no toxic solvent residue, products obtained by this method should provide minimal side effects in humans.

Pathogenesis of Osteoarthritis: possible target molecules for new therapeutic strategies.

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Background: Changes in the morphology and function of the chondrocytes during the Osteoarthritis (OA) progression can be related with the expression of molecules involved in the inflammatory process. Some of these molecules could be an important target to avoid, retard or even stop the progression of OA. Aims: 1) To describe the chondrocytes phenotype during early OA. 2) To identify possible target molecules involved in phenotype changes in OA chondrocytes. 3) To identify programmed cell death of OA chondrocytes.
Methods: The experimentally OA-induced model was accomplished by unilateral knee menisectomy and post-surgery training; normal rats were used as a control. Right femoral condyles were removed and processed for either electron microscopy (EM) or Immunohistochemistry (IHQ). Samples from rats with 1 to 5 training days (td) were used to evaluate ultrastructural changes by EM. Moreover, cartilage from rats with 3, 6 and 10 td were analyzed by IHQ for IL-1β, IL-10, TGF-β1 and TNF-α. Finally, programmed cell death was identified with TUNEL, caspasa 3 and LC3II at 20 and 45 td. Fluorescent signals were analyzed by confocal microscopy.
Results: EM revealed changes in the superficial zone chondrocytes, where cell phenotype changed from elongated to a rounded shape, in addition, the cells developed prominent endoplasmic reticulum (ER) with dilated cisterns and enhanced Golgi membranes. Expression of the proinflammatory cytokines IL-1β and TNF-α and began at 6 td, in the superficial zone and reached their highest levels at 10 td in all the cartilage zones. TGF-β1 showed diminished expression at 6 and 10 td. IL-10 was constant in all samples. Finally the cell death analysis, showed a co-expression of autophagic and apoptotic mechanisms.
Conclusions: Our results suggest that in early OA, chondrocytes changes its phenotype in order to synthesize proteins required for extracellular matrix (ECM) repairment. However, when its capacity is overwhelmed, chondrocytes begin the synthesis of catabolic molecules like IL-1β and TNF-α that stimulate an inflammatory process. Furthermore, the decrease of anti-inflammatory molecules could be involved in the beginning of the OA. Finally, the chondrocytes execute its own process of cell death, that include both autophagy and apoptosis.

Molecular Dynamics Simulations of human membrane transport proteins from the Major Facilitator Superfamily.

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The Major Facilitator Superfamily (MFS) constitutes the largest group of secondary active transporter proteins, with members ubiquitous in all kingdoms of life. Recent crystal structures of bacterial MFS proteins - including the Oxalate transporter, Glycerol-3-phosphate transporter,
Lactose permease and the multidrug resistance protein D - has revealed a common structural architecture. This MFS fold features two transmembrane domains composed of six helices each, connected with a cytosolic linker region of approximately 30 amino acids. Human MFS transporter structures, however, have not yet been obtained.

From a pharmaceutical standpoint, the human MFS transporters are of significant interest. This talk will present our studies on two human MFS proteins; (1) the glucose-6-phosphate transporter (G6PT), directly involved in the glycogen storage disease type 1b and (2) the vesicular glutamate transporter (VGLUT1) from the membranes of synaptic vesicles located in excitatory neural cells in the mammalian central nervous system. In the absence of structural data for these important human proteins, we have modelled atomic structures of G6PT and VGLUT1 from high-resolution crystal structures of bacterial homologues, and carried out extensive Molecular Dynamics simulations to better understand fundamental questions related to substrate binding, inhibition and conformational change of human MFS proteins.

Verapamil Reverts Acute Renal Functional Impairment Induced by Angiotensin II Converting Enzyme Inhibitors

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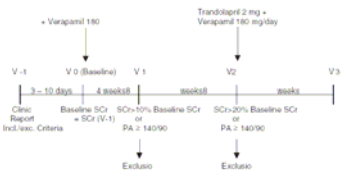
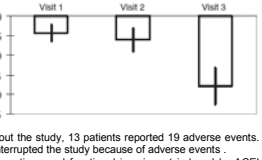
Background: Antihypertensive agents have been found effective in arresting glomerulosclerosis. Initially, it was thought that the healthy effect of these drugs was exclusively due to their hemodynamic effects. However, it has become clear that nonhemodynamic actions of these agents are an important component of their beneficial effects. Among thepharmac ological agents that may have a favorable
influence in the course of renal failure, angiotensin converting enzyme (ACE) inhibitors, and calcium channel blockers (CCB) have generated the most interest.
Angiotensin-converting enzyme inhibitors (ACEI) have proven to be effective drugs for the treatment of hypertension and represent a major therapeutic breakthrough in the management of hypertension and renal function preservation in diabetic and nondiabetic nephropathies. In some patients, ACEI may induce a rapid deterioration of renal function, assessed as an increase in serum creatinine (SCr), which can be reversed by withdrawing the drug.In these cases, maintaining the renal protection due to ACEI, instead of withdrawing these drugs could be desirable.
Calcium antagonists are a heterogeneous group of agents with diverse effects in terms of nephroprotection. Some of these differences relate to their effects on renal microcirculation. Dihydropyridine agents appear to act only on the afferent arteriole, increasing intraglomerular pressure, and albumin excretion rate. In contrast, nondihydropyridine agents like verapamil, may dilate efferent arterioles in addition to afferent arterioles and with normalization of the systemic blood pressure, verapamil may reduce intraglomerular pressure, and proteinuria. However, some other non hemodynamic protective effects of CCB could be explained by its capacity to inhibit the extracellular calcium influx, an important signal for the proliferative effect of mesangial cells mitogens, its influence in the decrease in mesangial entrapment of macromolecules and, possibly, its effect as free radical scavenger
Münter et al. describe that nephroprotective effects of ACEI/CCB combination can occur at doses, which do not significantly alter systemic blood pressure in the stroke-prone SHR. We have described that the combined therapy with these agents provide in the remnant kidney model a synergistic effect in preventing renal injury, independently of their effects on blood pressure.In a preliminary study, we have demonstrated in a small group of patients that nondihydropiridin (nonDHP) CCB are able to revert renal function reduction associated to ACEI treatment.
The main purpose of this study was to assess the efficacy and safety of low doses of verapamil (180 mg/day) added to the previous ACEI treatment for reverting decreased glomerular filtration rate observed in patients treated with ACEI. A secondary purpose was to test the ability of the fixed combination Verapamil-SR 180 mg plus Trandolapril 2 mg in attaining BP control and maintaining there renal function throughout the study.
Methods: This was a multicenter, nonrandomized, prospective, open study developed in five Spanish Hospitals. All patients were referred from the outpatients Departments of Internal Medicine and Nephrology. The Institutional Review Boards approved the study protocol and each patient entering the study signed a written informed consent.
Eligible patients presented a previous diagnosis of hypertension and an increase in SCr of 20% or 45 mmol/L from last values, in the course of ACEI treatment for more than four weeks. Exclusion criteria were renal insufficiency, defined as SCr >354 mmol/L (4 mg/dL), stroke, AMI in the last three months, unstable angina, cardiac failure, other causes of renal hypo-perfusion, or low volume syndrome such as dehydration, vomiting, diarrhea, laxative, and known hypersensitivity to verapamil.
In the selected group, a clinical evaluation was carried out, including clinical history, physical examination, BP measurement, ECG, and biochemistry (Visit 1). Patients fulfilling the inclusion/exclusion criteria were enrolled in the study. Three to ten days later a new BP measurement, serum, and urine creatinine and 24 h proteinuria were also measured (Visit 0 or Baseline) and creatinine clearance (CrCl) calculated. If the SCr was not higher than 10% respect to Visit 1, the patients were included in the study and Verapamil (180 mg/day) was added. Patients returned to the hospital after four weeks for new measurement of SCr and BP (Visit 1). If an increase on SCr superior to 10% respect to Visit 0 or BP was _140/90mm Hg was detected, ACEI was discontinued, and the patient withdrawn from the study. If not, treatment was continued for eight weeks. At this moment (Visit 2) patients were evaluated again. If SCr had increased more than 20% with respect to baseline, or BP was 140/90mmHg, the patients were withdrawn. If not, the patients were placed on the Trandolapril (2mg)-Verapamil (180 mg) association for eight more weeks.
Patients were followed until 20 weeks of follow-up were completed. All the patients were recommended to limit sodium intake.
At each study visit, blood pressure was measured three times at 2 min intervals after 10 min rest in the sitting position, using calibrated mercury sphygmomanometers. Blood pressure values were estimated as the mean of the three readings. The mean value obtained in Visit 0 was accepted as BP basal level. Biochemistry and SCr (Jaffe´ reaction) were determined in an automatic analyzer (Hitachi 747). Creatinine clearance was calculated as: urinary creatinine (mg/L) urine volume (mL)/serum creatinine (mg/L)/1.73m2.



All the basal variables were analyzed descriptively. Data are given as Mean ± standard deviation. Adverse events are codified according to the WHO Adverse Reaction Terminology List (WHO-ARTL). Repeated measurements ANOVA was used with an exploratory approach in order to test the following null hypotheses: changes of creatinine, systolic, and diastolic blood pressure, uric acid and potassium over time. The Wilcoxon test (two tailed) for paired samples was used to test the following hypotheses: changes of creatinine clearance and albuminuria between inclusion and week 12. Changes in creatinine between Visit 1 and inclusion, inclusion and week 12, inclusion and week 20, week 12, and week 20, historic control, and week 20. All the p-values are exploratory as type I error has not been adjusted for multiplicity.
Results: Forty-six patients, 23 female and 23 male, with a mean age of 54.9±7.3 years and BMI of 27.3±2.2 kg/m2, were included. Five of them (10.9%) had suffered from cerebrovascular disease, eight (17.4%) coronary heart disease, 24 (52%) had left ventricular hypertrophy on ECG or echocardiography, seven (15%) lower limbs peripheral vascular disease, and 35 (76%) some degree of retinopathy. Fourteen patients had been diagnosed from diabetes 4.5±8.0 years earlier (a median of 2.6 years): one was treated with diet, 12 with oral anti-diabetics drugs, and one with insulin. They were known hypertensive for a median of 4.5 years and all of them had been treated with ACEI for at least four weeks previous to SCr deterioration. Previously used ACEI were: enalapril in 12 patients, Lisinopril in nine, Cilazapril in seven, Perindopril in five, Quinapril in five, Ramipril in four, Trandolapril in two, and Captopril in two. Nine patients were withdrawn from the study, three at week four and six at week 12. Eight were withdrawn due to no BP control and no treatment compliance in one patient. In one patient, the reason was no BP control plus SCr increase>20%. A total number of 37 patients finalized the study.
The evolution of the main clinical and biochemical parameters is shown in the following Table.

	Lee control	Visit 1	Baseline	Visit 1 4th week	Visit 2 12th week	Visit 3 20th week (n = 37)	P
SBP (mmHg)	—	151.1 ± 13.2	148.5 ± 19.4	137.6 ± 3.4	137.0 ± 7.0	136.2 ± 5.7	0.0001*
DBP (mmHg)	—	95.5 ± 6.2	93.7 ± 6.4	86.2 ± 4.2	85.7 ± 6.1	84.4 ± 5.8	0.0001*
Scr (Cmc) (µmol/L)	97 ± 32	137 ± 46	136 ± 49	132 ± 47	126 ± 47	111 ± 31	0.0001*
Scr K^+ (mEq/L)	—	—	4.53 ± 0.42	—	4.43 ± 0.55	4.33 ± 0.25	0.0039*

*Repeated measurements ANOVA. SBP: Systolic blood pressure, DBP: Diastolic blood pressure, N = 46 except in Visit 3.



All the 46 patients were considered for the safety analysis. Throughout the study, 13 patients reported 19 adverse events. Symptoms were mild/moderate in intensity in all cases. Any patient interrupted the study because of adverse events .
Conclusions: The major finding of this study is the possibility of reverting renal functional impairment induced by ACEI treatment in hypertensive patients by adding verapamil without further modification in ACEI therapeutical regime. These patients had normal serum creatinine levels before ACEI treatment.
ACEI have been demonstrated to reduce morbidity and mortality in patients with heart failure. Renal impairment is the most important factor associated with prescription of lower-than-recommended doses. A recent review of 12 randomized clinical trials evaluating renal disease progression and ACEI-based therapy, shows that an acute increase of serum creatinine in the first two months usually follows the ACEI therapy in patients with preexisting renal failure. This is not our case, as the patients in our study had normal values of plasma creatinine before ACEI treatment. The review also shows that a limited elevation, of up to 30%, is strongly associated with long-term preservation of renal function when ACEI therapy is continued. In our study, we have observed increases in plasma creatinine up to 50% respect to pretreatment levels. These increases were completely reverted by adding verapamil to the ACEI treatment.
Some calcium antagonists seem to offer additional benefit in hypertensive patients when renal function is impaired, and may reduce proteinuria in hypertensive diabetic patients. The deterioration of renal function in patients receiving ACE-inhibitors seems to be due to a decrease in intraglomerular pressure, and, subsequently in the filtration net pressure. Thus, as angiotensin II has a predominant vasoconstrictor effect in the efferent arteriole, the decrease in angiotensin II due to ACE inhibition leads to an efferent arteriolar vasodilatation, and subsequently to a decrease in glomerular capillary pressure. This is one of the major beneficial effects of ACE inhibitors, as increased capillary pressure is a major cause of the progression of renal failure, but if the decrease on glomerular pressure is below normal values, glomerular filtration rate will be also decreased. However, it is difficult to accept this as the only cause of filtration rate decrease after ACEI treatment, because the autoregulation threshold is not always reached. If it is overpassed, an immediate deterioration in renal function must occurs at least in absence of decreased effective arterial volume, by far the commonest cause of acute rise in serum creatinine.
The additive effect of verapamil and ACEIs could be owing to that the two drug types protect the kidney by different mechanisms. Dworkin et al. have reported that either enalapril or nifedipine reduce renal injury in the remnant kidney model by different mechanisms: nifedipine reduces glomerular hypertrophy whereas enalapril reduces glomerular hypertension. Verapamil is able to diminish renal vasoconstriction mediated by BK receptor activation, prevent intrarenal vasoconstriction mediated by adenosine, and prevent from mesangial cells contraction and proliferation induced by several agents. Verapamil is also able to stimulate NO release.
The addition of verapamil to trandolapril treatment might promote other beneficial effects on renal structure and function. Thus, CCB are able to inhibit platelet aggregation, and this inhibition has been reported to ameliorate glomerular injury in rats with reduced renal mass. In addition, Harris et al. have shown that verapamil lessened oxygen consumption in the isolated, perfused remnant kidney and, therefore, it might ameliorate glomerular injury by reducing damaging oxygen radicals production rate.
The results of this study confirm our previous preliminary data suggesting a therapeutical approach, which allows revert the renal failure induced by ACEI treatment in hypertensive patients with previous normal renal function, maintaining this therapy, and adding verapamil 180 mg/day to the ACEI treatment. These findings are clinically relevant and will be of paramount interest for the patients in which ACE illicit a doubtless protection of target organs, even in nonhypertensive patients. The trandolapril-verapamil combination allows maintain an excellent BP control decreasing further the levels of serum creatinine.

Anti-tumour Vaccination in Advanced Malignancy with Class I & II hTERT Peptide-pulsed Dendritic Cells (DCs) Generates Suboptimal Antigen Specific CD8+Cytotoxic T Cell (CTL) Responses and Induces Regulatory T Cells in the Circulation

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Purpose: Human telomerase reverse transcriptase (hTERT) is expressed in >85% of human cancers and is becoming an increasingly popular molecular target for anticancer immunotherapy. We have previously shown that autologous DCs pulsed with class I epitopes of hTERT are capable of generating hTERT tetramer+, CD8+ CTLs in circulation of advanced cancer patients, albeit short-lived. By including CD4+cognate T cell help in the vaccination, through pulsing autologous monocyte-derived DCs with class II promiscuous hTERT peptides, we aimed to optimize the hTERT+, CD8+ CTL response in magnitude and duration.
Experimental Design: A Phase I clinical trial in advanced cancer patients was performed to evaluate the immunological and clinical impact of vaccinating (6 occasions, 2 to 3 weekly) advanced cancer patients with the HLA-A2-restricted class I hTERT epitopes I540 & I865 (n=10), with or without class II epitopes 766 & 675 (n=5), pulsed on autologous monocyte-derived *ex vivo* generated DCs.
Results: Peptide/MHC tetramer-CD8+ CTLs and CD4+CD25+ *foxp3*+ T cells were tracked sequentially through the vaccinations. Four patients with advanced cancer (prostate, renal, head & neck and colon) received an average (range) of 8.5 (6-12) vaccinations. . hTERT tetramer+, CD8+ CTLs were induced in 13/15 patients, with circulating tumour marker level reductions in 4/6 patients with prostate cancer. These were only short-lived and declined despite continuing vaccination. T regulatory cells (CD4+CD25+*foxp3*) tended to increased in the circulation during vaccination and showed a negative correlation with tetramer+ CD8+ CTL levels.
Conclusions: These results demonstrate that vaccination with autologous monocyte-derived DCs pulsed with hTERT class I & II peptides was unable to induce an optimal and sustained tetramer+, CD8+ CTL response but generated T regulatory suppressor cells in the circulation.

Hydrogels for Sustained and Selective Release of Diclofenac

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Background: The development of hydrophilic systems able to load hydrophobic drugs and to control the site and rate at which they are delivered is a target issue in pharmaceutical technology. The aim of our work was to prepare functionalized hydrogels based on semi-synthetic cellulose derivatives, acrylic copolymers or cyclodextrins, with high affinity for diclofenac for improving the loading and the release performance and even able to deliver the drug in response to certain stimuli.
Methods: The hydrogels were i) cross-linked cationic cellulose ethers with ethyleneglycol diglycidylethers (EGDE); ii) cross-linked cellulose ethers/ cyclodextrins with EGDE; iii) copolymerized hydroxyethylmethacrylate (HEMA) with aminopropyl methacrylamide (AMPA) or vinylpyridine (VP); and iii) interpenetrated networks of N-isopropylacrylamide (NIPA) and chitosan.
Results: Cross-linked cationic cellulose networks loaded 250 mg diclofenac per gram and selectively released the drug at pH>7, being useful for specialized intestinal delivery. A novel cross-linking method to prepare cyclodextrin hydrogels led to systems that hosted the drug in the cyclodextrin cavities (100 mg/g) and sustained the delivery for 8 hours. Poly(HEMA-co-APMA) and poly(HEMA-co-VP) loaded 15 mg/g and did not release the drug in water but in the presence of ions; the release being sustained for several days. Such hydrogels display features particularly adequate for the development of medicated soft contact lenses. Interpenetrating NIPA/chitosan networks showed temperature- and pH-sensitive loading and release behavior. The IPNs had a notably greater affinity for diclofenac (18 mg/g) than the pure PNIPA hydrogel and were able to sustain the drug release for more than 8 h in 0.9% NaCl solutions or pH 8 phosphate buffer. The IPNs with low chitosan postcross-linking degree showed high temperature-sensitive release patterns.
Conclusions: Loading of diclofenac and its release pattern can be tunned designing tailored hydrogels in which functional elements performing as high affinity binding points and environment-responsive sensors are combined. These functionalized hydrogels may be particularly useful for site-specific drug delivery.

Authors disclosure statement:
Some information described in this abstract is the subject of patent applications filed by the University of Santiago de Compostela (WO 2006/089993; ES 200802364).

Clinical importance of drug resistance in antiviral therapy for Hepatitis B Infection

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Background: Hepatitis B Virus (HBV) is a unique DNA virus which replicates through an RNA intermediate, lacks proof reading ability, has high viral replication rate. This leads to random mutations with amino acid substitution in reverse transcriptase region. In clinical parlance antiviral drug resistance is defined as selection of variants bearing amino acid substitution confirming reduced susceptibility to drug those results in primary or secondary treatment failure. For the management of HBV infection in addition to interferon/Peg Interferon/Thymosin alpha. 4 oral antiviral like lamivudine, adefovir, entecavir, Telbuvidine are licensed and Tenofovir and emcetarabine are licensed for HIV & HBV coinfection. Success rate of these antiviral agents do not exceeded more than 30 to 40% in long term treatment and with prolonged therapy, menace of antiviral resistance exists. Clinical consequence of resistance are decreased HBeAg clearance, reversal of histological improvement, increased rate of disease progression, ,clinical decompensation or even death in patients with cirrhosis, risk of graft loss and death in liver transplant recipients, transmission of drug resistance strain and vaccine failure mutation.
Methods: We analyzed real life data on our patients receiving long term Lamivudine treatment and development of resistance clinical consequences and their management...
Results: Our study included 82 patients (male 66, Age range 5-85 years). Of the 82 patients 50 patients were HBeAg +ve. These patients received mean duration of Lamivudine treatment 32.44 months. 17 out of 50 (34%) developed resistance to Lamivudine, 32 patients were HBeAg –ve, Mean duration of treatment with Lamivudine was 28 months. 8 out 32 (25%) developed Lamuvidine resistance. The presentation of Lamivudine resistance was clinical decompensation 3, sero-reversion 7, flare of liver enzymes 8, and increased viral load 5. All the patients who developed resistance were treated with addition of Adefovir to Lamivudine. Mean period of 8.5 months of follow up; 2 patients died dues to decompensation, remaining patients are stable with normalized liver function
Conclusion: Antiviral drug resistance is a major problem in management of chronic HBV infection. Combining second drug with no cross resistance at appropriate time seem to be best policy currently.

The F1-V Plague Vaccine Can Activate the Innate Immune Response Through Toll-Like Receptor2 and 4 but Does Not Need Them for an Antibody Response to the Vaccine

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Background: A new recombinant fusion protein F1-V is in advanced development for preventing plague caused by *Yersinia pestis*. F1 is a capsular protein, and V is a low calcium response (Lcr) protein or V-antigen. V-antigen has been reported to activate Toll-like receptor (TLR) 2. We hypothesized that activating the innate immune system through TLRs is required for an antibody response to the F1-V vaccine.
Methods: We evaluated the interaction of V-antigen and F1-V with human embryonic kidney (HEK)-293 cells expressing TLR2 or TLR4 (InvivoGen). Further, we examined the antibody response, the proliferative response, and cytokine expression by splenocytes from F1-V-vaccinated TLR 2, TLR 4, TLR2/4, or myeloid differentiation factor 88 (MyD88) knock-out (KO) mice. Activation of TLR 2 and 4 in HEK-293 cells was measured as per manufacturer's instructions. Mice were vaccinated subcutaneously twice (0 and 28 days) with F1-V (1 – 2 *u*g) formulated with Alhydrogel. Serum and spleens were removed from anesthetized mice 21 days after the boost. The antibody response to the vaccine was measured by end-point ELISA. Cytokine expression by antigen-stimulated splenocytes were measured by BD FACSArray analysis after 40 h. Proliferation of splenocytes was determined by the amount of ^{3}H-thymidine incorporated after an additional 18 h incubation.
Results: TLR2 and TLR4 HEK-293 cell lines were weakly activated by V-antigen but strongly by the F1-V vaccine. These antigens activated no other TLR cell lines. Wild- type, TLR2, and TLR4 mutant mice vaccinated with the F1-V vaccine all appeared to respond similarly to the prime and boost administration of the vaccine. This included the IgG1 and IgG2a isotype response to the vaccine. Furthermore, the antibody response in MyD88 KO mice was similar to that in wild-type mice. The proliferative response and cytokine expression was partially affected in the TLR2 KO but was essentially background level in the TLR4 KO, TLR2/4 KO, and MyD88 KO mice.
Conclusion: 1. TLR2 and TLR4 mediated innate immune responses are not required for an antibody response to the plague vaccine. 2. MyD88 is also not required for an antibody response to the vaccine. 3. Cellular immune responses to the vaccine appear to be partially dependent on TLR2 but appear to be completely dependent on TLR4.

Pharmacokinetic study of rivastigmine in Iranian healthy subjects following 3 and 4.5 mg dosing using a simple and sensitive HPLC-UV method

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Background: Rivastigmine is relatively new drug and the evaluation of its pharmacokinetic properties in different ethnic populations is important to optimize the dosage regimens. For the pharmacokinetic study of rivastigmine, a simple and rapid but also a highly sensitive and selective bioanalytical assay method should be available. Aims: 1) To develop and validate a sensitive and selective analytical method for rivastigmine assay in plasma 2) To perform the pharmacokinetic study in Iranian healthy subjects 3) To compare the pharmacokinetics of rivastigmine following 3 and 4.5 mg dosing.
Methods: A simple and reproducible HPLC method with spectrophotometric detection at 200 nm was developed and validated for the determination of rivastigmine in human plasma. The assay was used for pharmacokinetic study of rivastigmine capsules in healthy Iranian subjects following 3 and 4.5 mg dosing. 23 healthy and fasted volunteers participated in both groups. Food and drinks were not allowed until 3 h after ingestion of the capsules. Multiple blood samples (5 ml) were collected before and 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6 and 8 h post-dosing. A non-compartmental analysis was used in the data processing.
Results: In HPLC analysis**,** it was founded that in addition to reversed-phase retention, other retention mechanisms such as hydrogen-binding or ion-exchange are probably involved in the chromatogeraphic behaviour of rivastigmine. Triethylamine should not be used in the mobile phase, while an acidic mobile phase and chromatography at high temperatures can increase theoritical plates for rivastigmine. A selective extraction of rivastigmine from plasma was obtained using 1-butanol/n-hexane (2:98, v/v) and back extraction into diluted acetic acid. The newly developed HPLC-UV method had an limit of quantification (LOQ) of 0.5 ng/ml, which is comparable to LOQ of 0.2 ng/ml obtained by current LC/MS methods. The pharmacokinetic studies showed that rivastigmine has a rapid oral absorption with a large inter-subjects variations. The mean values of maximum plasma concentration (Cmax), time to Cmax (tmax), area under the plasma concentration-time curve from time 0 to 8 hours (AUC8) and from time 0 to infinity (AUC∞), and plasma half-life following administration of the rivastigmine at 3 mg dosing were 6.27 ng/ml, 0.98 h, 11.95 ngh/ml, 12.79 ngh/ml and 1.11 h, respectively, and for 4.5 mg dosing 10.74 ng/ml, 0.91 h, 21.60 ngh/ml, 22.98 ngh/ml and 1.22 h, respectively.
Conclusions: For the first time, a highly sensitive HPLC-UV method was developed and validated for rivastigmine assay in plasma. Pharmacokinetics of rivastigmine in Iranian healthy subjects were comparable with results obtained in other ethnic populations. As reported by others, the oral bioavailability of rivastigmine increased with dose.

Antibiotic resistance: what can we learn from evolution?

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Background: The brilliant "Zauberkugeln" (Magic bullets) idea of Paul Ehrlich was a major breakthrough in treating infectious diseases. It opened the whole new era in medicine and led to the discovery of many antibiotics that saved millions of lives. Despite the considerable success, however, bacteria invented various shields thus compromising the magic power of antibiotics. The number of pathogens that learned how to dodge these bullets is increasing and the question is how they acquired these properties? Aim: reconstruction of evolutionary history of selected antibiotic resistance genes to answer this question.
Methods: Four sets of genes encoding resistance to tetracyclines, macrolides, vancomycin and fluoroquinolones were chosen for this analysis: 1) tetracycline resistance genes, encoding ribosomal protection proteins; 2) the *erm* genes encoding enzymes that methylate the specific adenine residue in the 23S rRNA molecule; 3) the vancomycin resistance gene cluster represented by the concatenated set of *vanHAX; and* 4) the *qnr* genes conferring resistance to fluoroquinolones. The sequences were downloaded from GenBank and aligned using ClustalX ver. 1.83. Maximum-likelihood and Bayesian inference were used to reveal the evolutionary history of these genes.
Results: Phylogenetic reconstruction suggested a long evolutionary history of diversification of antibiotic resistance genes that began well before the "antibiotic era". There is no indication that lateral gene transfer from antibiotic-producing bacteria has played any significant role in shaping the pool of antibiotic resistance genes in pathogenic and commensal bacteria. The primary antibiotic resistance gene pool originated and diversified within the environmental bacterial communities, from which the genes were mobilized and penetrated into taxonomically and ecologically distant bacterial populations, including pathogens.
Conclusions: Enormous metabolic diversity of bacteria allows them to come up with protection mechanisms even against novel antibiotics. To preserve the magic of novel antibiotic bullets we have to pay more attention to the pool of antibiotic resistance genes in the environment and carefully monitor the possible movement of such genes into commensal and pathogenic bacteria.

Activity of Antimalarial Constituents of *Spathodea campanulata*

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Background: The search for antimalaria drugs is a continuous one because of the devastating effect of the disease. Aim: To determine the antimalarial properties of *Spathodea campanulata*.
Methods: This study included 165 Swiss albino mice in Fink and Kretschmar's, and Rane *in vivo* tests. Five mice were used per treatment, weight range: 18-22g. In the Fink and Kretschmar's test, each mouse was inoculated with *Plasmodium berghei* berghei and treated post-infection subcutaneously once daily for 4 days with plant constituents, chloroquine or blank control. The % parasitaemia was evaluated on the fifth day post-infection. In the Rane test, the mice were treated with the drugs once daily for 4 days starting 3 days post-infection. The % parasitaemia for each mouse was determined for 5 days starting from the fouth day post-infection. The active constituents of the plants were isolated by column chromatography and characterised.
Results: Antimalarial principles of stem bark were ursolic acid, tomentosolic acid, 20β-hydroxyursolic acid and caffeic acid from leaves. In Fink and Kretschmar's test ursolic acid at 15-60 $mgkg^{-1}day^{-1}$ produced 34-97% suppresion of parasitaemia and mean survival period of 13-25 days. Tomentosolic acid at 10-80 $mgkg^{-1}day^{-1}$ produced 35-82% suppresion of parasitaemia and mean survival period of 10-19 days. 20β-hydroxyursolic acid at 20-80$mgkg^{-1}day^{-1}$ produced 11-53% suppresion of parasitaemia and mean survival period of 8-13 days. The aqueous leaf extract at 50-400 $mgkg^{-1}day^{-1}$ produced 0-74% suppresion of parasitaemia. Chloroquine at 10 $mgkg^{-1}day^{-1}$ produced 98% suppresion of parasitaemia and mean survival period of 26 days.
In Rane test the aqueus leaf extract at 50-400 $mgkg^{-1}day^{-1}$ produced mean survival time of 11-15 days. Ursolic acid at 15-60 $mgkg^{-1}day^{-1}$ produced mean survival period of 9-24 days. 20β-hydroxyursolic acid at 20-80 $mgkg^{-1}day^{-1}$ produced mean survival period of 6-16 days. Tomentosolic acid at 5-40 $mgkg^{-1}day^{-1}$ produced mean survival period of 9-18 days. Blank control gave mean survival period of 7 days in both tests.
Conclusions: 1) The antimalarial principles of *Spathodea campanulata* demonstrated significant schizontocidal properties, the activity of chloroquine was however superior to any of them. 2) The activity of the antimalarial principles provides the scientific basis for the use of *Spathodea campanulata* in the management of malaria in traditional medicine.

Metalloantibiotics: Synthesis and Antibacterial Activity of Metal(II) Complexes Containing Cephalosporin and Sulfathiazole

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Background: The interaction of antibiotics with main and transition metal ions has attracted our attention and compelled us to combine their chemistry in order to improve the stability and efficiency of antibiotics. Thus, designing a new area of research in the synthesis of stable metalloantibiotic compounds that may be used as active drugs working effectively against antibiotic resistance species.
Methods: The metal(II) complexes were prepared by mixing clear solutions of the appropriate cephalosporin sodium salt (1 mmol) and $NiCl_2.6H_2O$ or $CuCl_2$ metal salts (1 mmol) in distilled water (10 mL) and sulfathiazole (1 mmol) in EtOH (10 mL). The reaction mixture was then stirred at room temperature for 12 h. and green precipitates formed. The precipitated complexes were filtered off, washed with water, MeOH and ether and dried under reduced pressure at room temperature. Yield 55-65%. No attempts to use different molar ratios to prepare the complexes were made. Antibacterial activities were tested using the paper disc diffusion method. The chosen strains were *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 11775, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 23357, *Salmonella enteritidis* CDC 64 and *Bacillus subtilis* ATCC 6051.
Results: Nickel(II) and copper(II) react with cephalosporins plus sulfathiazole (Hstz) to form the following mixed ligand complexes: $[M(cefazol)(stz)(H_2O)]$, $[M(cephalot)(stz)(H_2O)_2]$, [M(cefotax)(stz)], [M(ceftria)(Hstz)] and [M(cefepime)(stz)]Cl (Hcefazol = cefazolin, Hcephalot = cephalothin, Hcefotax = cefotaxime, H_2ceftria = ceftriaxone) which were characterized by physicochemical and spectroscopic methods. Their spectra indicated that most the cephalosporins are acting as a monoanionic multidentate chelating agents, the exception being ceftriaxone which is dianionic. The complexes are insoluble in water and common organic solvents and probably have polymeric structures. They have been screened for antibacterial activity in DMSO solutions against several bacteria, and the results are compared with the activity of cephalosporins. The [M(cefepime)(stz)]Cl complexes showed better activity than free cefepime against all bacteria strains, including against *P. aeruginosa* and *S. aureus* where cefepime is inactive.
Conclusions: The synthesized compounds showed antibacterial properties. In comparison, the copper(II) and nickel(II) complexes containing cefepime plus stz showed better activity against several bacterial strains than the cefepime, thus introducing a novel class of metal-based bactericidal agents.

Production, isolation, partial characterization and antimicrobial spectrum of a novel bacteriocin produced by a Lactobacillus plantarum strain in fermentation

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Background: Fermentation broths (in MRS) of an isolated Lactobacillus plantarum strain exhibited strong antimicrobial activity against common food spoilage and also food born pathogens. Antimicrobial activity was assessed in the agar diffusion assay using a total of 9 indicator food-grade bacteria. The antimicrobial activity appeared to be higher against closely related species and Lactobacillus curvatus was chosen as the most suitable among the tested microorganisms to serve as indicator for the quantification of the produced bacteriocin.The exhibited antimicrobial activity was due to the production of a proteinaceus compound, a bacterocin, most of which appears to be cell-associated.
Methods: A number of mechanical and physicochemical treatments were applied to washed cells in attempting to solubilize the bacteriocin. The most convenient method for extraction was centrifugation at 20200 rcf for 10 minutes at 4oC . Repeated tricine - SDS - PAGE electrophoresis of samples taken at various fermentation time-points showed that bonds as ~ 30kDa were of interest.The positon was finally determined by overlying the gel with MRS agar in which L. curvatus was embedded.
Results: The M.W. of the bacteriocin was estimated at 30kDa.The isolated bacteriocin lost its activity after treatment with lipase and ?-chymothrypsin, but retained activity following proteolytic treatment. The stability of bacteriocin was studied over a range of pHs,T and mechanical stresses.
Conclusions: It appeared to be heat labile, a characteristic indicative, along with M.W. and the sensibility to lipase and ?-chymothrypsin, of a certain category of antimicrobial peptides, the class IV of bacteriocins produced by lactic acid bacteria. Fermentation kinetics studies performed in a stirred tank bioreactor showed that the production of the bacteriocin was not associated to growth, but it was rather formed as a secondary metabolite.

Systems-directed targeted therapies in metastatic tumors: Equitable to reductionist therapy approaches?

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Introduction: As we consider the exchange of information between tumor, adjacent stroma cells, and cells of the involved organ from a **systems perspective,** we may disregard operational prerequisites that a combination of activities triggered by specific action systems must be intended by single participating pathophysiological mechanisms, such as inflammation, angiogenesis, Warburg effect, immune response, extracellular matrix remodeling, proliferation, apoptosis, coagulation. Activities that seem to be operationally induced by the division of function present itself from a systems perspective as an **enhancement of complexity.** We hypothesized, that tumor systems-directed therapies might have the capability to use **aggregated action effects,** as adjustable sizes to therapeutically modulate the tumor systems' stability, homeostasis, and robustness.
Methods: We performed a retrospective analysis of recently published data on 278 patients with advanced and heavily pre-treated (10% to 63%) vascular sarcoma, melanoma, renal clear cell, cholangiocellular, mucoepidermoid, and hepatocellular carcinoma, hormone-refractory prostate cancer, glioblastoma, and multivisceral Langerhans' cell histiocytosis enrolled in nine multi-center phase II trials (13 centers). Each patient received a multi-targeted systems-directed therapy that consisted of metronomic low-dose chemotherapy, a COX-2 inhibitor, combined with one or two transcription modulators, pioglitazone +/- dexamethason or IFN-alpha.
Results: These treatment schedules may attenuate the metastatic potential, tumor-associated inflammation, may exert site-specific activities, and induce long-term disease stabilization followed by prolonged objective response (3% to 48%) despite poor monoactivity of the respective drugs. Progression-free survival (PFS) data are comparable with those of reductionist-designed standard first-line therapies targeting preferably tumor cell-specific pathways.
Conclusions: Differential response patterns indicate the therapies' systems biological activity. Structured systems-directed therapies in metastatic cancer, targeting amongst others inflammation and neoangiogenesis, may break through the barrier of complexity of tumor-stroma-interactions, and get a source for detecting topologies of tumor-associated aggregated action effects as adjustable sizes for targeted biomodulatory therapies. Biomodulation of systems biological processes facilitates comparatively high efficacy at moderate toxicity.

A diarylquinoline targeting the energy supply of M. tuberculosis

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We discovered a diarylquinoline (TMC207 or R207910) with potent bactericidal activity against drug-sensitive and drug-resistant Mycobacterium tuberculosis. Whole genome sequencing of resistant mutants suggested that the drug targets the energy supply of mycobacteria by inhibition of the ATP synthase. The oligomeric subunit c (AtpE) of ATP synthase was validated as the target by genetic, biochemical and binding assays. Unlike other TB drugs, TMC207 is equally active against growing and dormant TB bacilli, making it a good candidate for shortening TB therapy.

In mice, four weeks of TMC207 monotherapy exceeds the bactericidal activities of isoniazid and rifampin by at least 1 log unit. Substitution of rifampin, isoniazid, or pyrazinamide (the World Health Organization's first-line treatment regimen) with TMC207 accelerated bactericidal activity, leading to complete culture conversion after 2 months of treatment in some combinations, against 5 months for the standard regimen. Four months of treatment with rifampin + pyrazinamide + TMC207 yielded the same relapse rate as six months of the standard regimen. Similar improvements were observed when TMC207 was combined with drugs to treat MDR-TB, suggesting that use of TMC207 may also significantly reduce the duration of treatment of MDR-TB.

The bactericidal activity of TMC207 was confirmed in patients in a one week early bactericidal activity trial and the drug is now being investigated in a phase 2 trial in MDR TB patients

The Chemical Interaction of the Anticancer Drugs Irinotecan-HCl and Epirubicin-HCl in the Same Infusion Solution

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Background: Because the administration of infusions sometimes needs a long period of time in combination chemotherapy, it would appear that the best and easiest method is to give more than one drug in the same infusion solution. Because of this, investigation of the chemical compatibilities of chemotherapeutic drug combinations given in the same infusion solution before clinical studies is quite important. A new combination epirubicin and irinotecan were chosen for investigation of chemical incompatibility, in order to search the applicability of these two drugs in the same infusion solution in a combination chemotherapy.
Methods: Visual compatibility was assessed by means of effervescence, colour change, precipitation and pH change after the drugs had been injected into same infusion solution. Chemical interaction was further investigated quantitatively using a spectrophotometric method in infusion solution in clinical concentrations. The molar ratio for the reaction to proceed was also determined. All the interaction study was repeated in pharmaceutical forms, imitating a real application.
Results: No sign of incompatibility was observed upon visual examination. But a chemical incompatibility was observed in the UV spectra of the drug mixtures. The molar ratio of epirubicin-HCl/irinotecan-HCl at which the interaction reached a maximum was found to be 2:1. The chemical interaction occurred immediately after admixing and no visual or spectral change was noticed for 24 h after the interaction had occurred.
Conclusions: These drugs are chemically incompatible. The positive or negative contribution of such chemical interactions to the pharmacological effect of the combination might be of importance and while the applicability of these two drugs in combination is investigated in further pharmacological studies, their chemical interaction should also be a consideration.

A Potential Tumor Cell-Penetrating Peptide, CRGDCF(K[H-]KKK)$_6$, for the Delivery of Antisense and siRNA Oligonucleotides

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Background: We have previously developed a tumor cell-penetrating peptide (cRGD-hK) of 36 amino acid residues, which comprises a cyclic RGD motif for tumor homing and cell internalization, a DNA- or RNA-binding oligolysine, and histidyl residues to facilitate the delivery into the cytosol. The length of antisense or siRNA oligonucleotides is thought to match that of the oligolysine, presuming that both form complexes electrostatically in a 1:1 molar ratio (Figure). Then, we have been assessing the possibility of such complexes as antineoplastic agents.
Methods: 1) With luciferase expression plasmids as a reporter, we tested the functional potency of elements of cRGD-hK in cancer cells. 2) The effects of the complexes of cRGD-hK with antisense phosphorothioate DNA and siRNA corresponding to the luciferase gene were compared *in vitro*. 3) Using siRNA corresponding to the c-*raf* gene, we assessed the effects of cRGD-hK/siRNA complexes on intracellular c-Raf protein levels of pancreatic cancer cells and on the tumor growth in nude mice.
Results: 1) The three elements of cRDG-hK were indicated to function as expected, by using bafilomycin A_1 and cycloRGDfV. 2) The gene-silencing effect of the complexes with siRNAs was greater than that with antisense phosphorothioate DNA. 3) The peptide/siRNA complexes lowered intracellular c-Raf protein levels of cultured cells at less than 500 nM for 48h incubation. When tumor-bearing nude mice were intraperitoneally administered with the complexes (5 μg RNAs three times a week for 4 weeks), the tumor growth was found to be significantly (P<0.05) inhibited in the third and fourth week.
Conclusions: It is suggested that the cRGD-hK could function as a tumor cell-penetrating peptide for the delivery of siRNA and antisense oligonucleotides, but further studies are needed.

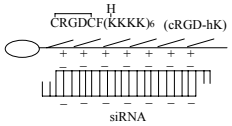

Fig. Schematic drawing of a putative complex of the tumor cell-penetrating peptide (cRGD-hK) and small interfering RNA duplex (siRNA).

Preoperative use of Analgesia in Appendicites

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Background: Appendicitis is a common cause of acute abdominal pain, early analgesia was considered previously as it could mask physical signs and hence delay the diagnosis and surgical intervention, now this has been challenged
Methods: A prospective, experimental study had been carried out in Ibn Sinna general Hospital from November 2006 to March 2007, our aim was to determine the influence of Diclofenac sodium DS(voltaren) on masking the diagnosis of acute appendicitis.
The data collected by using well designed questionnaire with observation during the period of admission before the operation.
Results: The study includes 80 patients (40 as cases and 40 as controls) and The result revealed that most of the symptoms (fever, anorexia, nausea and vomiting) and sings (tenderness, obturator and Psoas signs, local guarding and rigidity), in addition to the rate of perforation and the vital sign were not hidden by DS(Voltaren) with P value >0.05.while other symptoms (pain) and signs (rebound tenderness, Rovsing and pointing) had been hindered by the use of DS (voltaren). The most common presenting symptom in placebo and DS group was pain (100%) which showed a marked decrease in severity in those who received voltaren as analgesia (72.2%)
Conclusion: Some of the symptoms and signs of acute appendicitis were masked by the use of analgesia; while others were not .and overall Diclfenac sodium did not influence the decision of diagnosis or the management of acute appendicitis.

Single Chain Antibodies (ScFvs) and Immunoconjugates: Computational and Functional Approaches

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Background: ScFvs in which the variable heavy and light chains are connected by a peptide linker maintain the binding specificity and affinity of the parental antibody IgG. ScFvs coupled to highly toxic molecules (immunoconjugates) are currently being developed for cancer therapy. Aims: 1) To assess the effects of specific mutations on the stability, structure and dynamics of the scFv antigen binding site. 2) To develop an *in silico* procedure for evaluating physicochemical properties of two tumor-targeting anti-HER2 immunoconjugates. 3) To propose plant-based expression systems for high-level scFv production.
Methods: This study included four scFvs (scFv(F8), scFv(ADDLs), scFv(FR5), scFv(800E6)) and two tumor-targeting anti-HER2 immunoconjugates (scFv(FR5)-ETA, scFv(800E6)-ETA). For the computational procedures all the antibody structures were derived by homology modeling and assessed by molecular dynamics (MD) simulations. As regards the experimental section tobacco plants were transformed for stable and transient expressions. Specific expression vectors containing the gene encoding for the scFvs of interest were used. Transgenic plants were cultivated also in hydroponic and aeroponic systems. DNA, RNA and protein analyses were performed in leaves, roots and root exudations.
Results: Structural and MD analysis indicated a strong correspondence between structurally–determined flexibility of the binding site with the different functional behaviors proved by the wild-type and its mutants. Computational analysis of anti-HER2 immunoconjugates showed that the presence of a toxin does not significantly affect the major physicochemical parameters and their structure. The highest level of scFvs expression was observed in roots.
Conclusions: 1) The computational approaches represented a good tool for structure-based design of antibody-binding site, for analyzing physicochemical properties of immunoconjugates and for predicting the effects of the linked toxin on structure, dynamics and functionality of the antibodies. 2) The proposed plant-based expression system seems to represent a promising tool for a large-scale scFv production.

Exercise as a Modality to Identify Therapeutic Molecules for Treatment & Prevention of Cancer-Associated Cachexia: Possible Enhancement of Anti-inflammatory Cytokines Through an Intermittant Activation of the Stress-Response Pathways

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Background: Repeated exercise is well-known to reduce risk for cancer, cardiovascular disease, type II diabetes, and a variety of neurological disorders; a magic bullet if there ever was one! Cachexia affects approximately 50% of all cancer patients and is characterized by weakness, fatigue, anorexia, adipose and skeletal muscle atrophy, insulin resistance, and impaired immune function; a condition desparately in need of a magic bullet! Cachexia appears to be associated with elevated levels of PIF, TNF-α, IL-1, IL-6, and Interferon-γ. Exercise induces the production of sTNFr, IL-1ra, and IL-10 (anti-inflammatory cytokines). Aim: To test the hypothesis that activation of stress-response pathways may be involved in the protective effect of exercise.
Methods: Rats were familiarized with a rodent treadmill on four separate days over a period of two weeks and then forced to run for 60 minutes at a speed of 27 m/min (a moderately hard workload for untrained rats). Three animals were killed at each time point: 0, 15, 30, 60, 90, 120, 180, 240, and 300 minutes after the start of the exercise. Lungs from 3 animals at each time point were pooled and nuclei prepared by Dounce homogenization and differential centrifugation. Nuclear proteins were analyzed by western blot using anti cJun/AP1 monoclonal antibody (Ab-3, Oncogene Research Products).
Results: Jun binding was appearent at 4 hours and one hour later was undetectable. This indicates that a 60 minute bout of running exercise is sufficient to enhance jun content but that this effect is of a relatively short duration.
Conclusions: Based on these preliminary results the possibility exists that a transient exercise/stress-mediated activation of the MAPK and/or JNK-MAPK pathways (possibly through enhanced Ca^{++} and ROS) is responsible, in part, for the anti-inflammatory effects of exercise as well as the enhanced activity of antioxidant enzymes and phase II enzymes previously observed by this lab. Because skeletal muscle is far more metabolically active than lung during exercise, one might expect that the degree of an exercise-induced activation of AP-1 in muscle would be greater than that of lung. Development of drugs which mimic this activation profile should produce similar benefits.

Magic Bullets in Removing *Enterococcus faecalis* Biofilms.

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Background: *Enterococcus faecalis* is the most common and, occasionally, the only single isolated bacterium from root canals of teeth with persistent periapical periodontitis. Its inherent antimicrobial resistance and ability to adapt to harsh environmental changes make *E. faecalis* responsible for many endodontic failures; moreover, such adverse conditions may favour the growth of this bacterium as a biofilm in root canal walls.
The elimination and/or control of *E. faecalis* biofilms is a goal in root canal therapy. Several irrigating solutions are used during the endodontic treatment; some of them have been widely tested against planktonic bacteria. Because of the high resistance of the biofilms to the endodontic irrigants, the aim of our study was to evaluate the effectiveness of four irrigating solutions used in root canal teeth against *E. faecalis* biofilms.
Methods: Four irrigants - sodium hypochlorite, chlorhexidine, ethylene-diaminetetraacetic acid (EDTA) and citric acid - were tested at 1, 5 and 10 min of exposure to biofilms of *E. faecalis* ATCC 29212. The biofilms were grown aerobically in the MBEC™ high-throughput device for 24h at 37ºC. They were exposed to ten serial twofold dilutions of each irrigating solution. The antibacterial activity of the root canal irrigants was evaluated by determining the viable cell counts and log killing of *E. faecalis* biofilm cultures. A concentration of an irrigant was considered to be effective when it produced a reduction of ≥ 5 logarithmic units.
Results: Sodium hypochlorite was the most effective solution at any dilution and time of exposure tested. Chlorhexidine and citric acid solutions showed less antibacterial activity and needed more time to kill *E. faecalis* biofilms. EDTA solution lacks antibacterial activity against *E. faecalis* biofilms even after 10 min contact time.
Conclusions: Sodium hypochlorite should be elected as the best irrigating solution as it showed the highest effectiveness against *E. faecalis* biofilms.

Vaccination with Recombinant MHV68 Producing IFNα Effectively Protects Mice Against Infection With Wild Type MHV-68 and Dramatically Reduces the Establishment of Long-term Spleen Latency.

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Background: Human gammaherpesviruses such as Epstein-Barr Virus cause lifelong infections and associated diseases, by virtue of their ability to establish latent infection.
Mice infected with murine herpesvirus 68 (MHV-68) represent a versatile experimental setting to study the biology of gammaherpesviruses and to test vaccination strategies against them.
We recently observed that a clone of recombinant MHV-68 carrying the mouse IFNα1 gene (MHV-68mIFNα1) shows a significant *in vivo* attenuation, which is mediated by the cytokine released during the course of the infection, and affects both the acute replication and spleen latency.
Methods: C57 BL/6 mice received two intranasal (i.n.) administrations of 10^5 earstwhile pfu of psoralen-UV-inactivated wt or recombinant IFNα1-producing MHV68. A group of mice received i.n. 10^5 pfu of live-attenuated MHV68m IFNα1 as a vaccine. Four weeks later, vaccinated and a group of unvaccinated mice were infected (i.n.) with 4 x 10^5 PFU of MHV-68.
Virus titres in the lungs and spleen latency were measured by plaque and infectious centre assay respectively. Virus non-specific B-cell activation was assessed by FACS. Molecular analysis of the viral genomes harboured in mice spleens cells was performed by real time PCR. An ELISA was used to quantify the anti-MHV68 humoral immune response.
Results: Mice vaccinated with live-attenuated or partially inactivated MHV-68mIFNα1 were protected against the challenge with wt MHV-68 in terms of acute replication and long-term latency. This protection was associated with a significant virus-specific IgG antibody response.
Conclusions: IFNα1, produced at the site of the infection by the vaccinating virus, acted as an adjuvant in stimulating an anti-MHV68 immune response effective in eliciting protection against all phases of MHV68 infection. We believe that the ability of MHV-68mIFNα1 to produce IFNα1, thus efficiently stimulating the immune system whenever it reactivates from latency, makes this recombinant virus a safer live-attenuated vaccine as compared to the latency-deficient clones previously described by others.

Remifentanyl: How it Relives Human and Earthly Pain, and New Perspectives.

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Background: Remifentanyl use has become a standard analgesic therapy during and after low-high complexity surgeries. Its indications have extended beyond surgery rooms and today, it is one of the drugs that has importantly changed the way of administrating anesthesia for a considerable number of procedures specially for those that are ambulatory (a-day surgery). However it is unknown what it´s impact will be over the emission of halogenated anesthetics which may be the origin of organic-halogenated pollutants (OHP) that are released into the atmosphere, resulting in ozone layer damage and more over these compounds are recognized as neurotoxic and endocrine modulators. Around the world there are many institutions where it is not possible to administer total endovenous anesthesia (TIVA) due to economical implications. Our objective was to analyze the impact of the use of remifentanyl during surgery over the quantity of halogenated anesthetics, using intermediate infusions of remifentanyl (0,15-0.25 mcg/kg/min).
Methods: Over a period of one year an initial prospective study was undertaken to identify mean plasma concentrations of remifentanyl for a target biespectral index (BIS) of 40-50, in a hispanic population at a level 4 hospital, based on the pharmacokinetic model described by Minto et al. The second stage of the study was to identify the total amount of halogenated gases consumed during this period comparing it to the consumption from the previous year. Variables are reported as number of patients, medians and ranges, means and SD. Nominal data were analyzed using the exact Fisher test. Ordinal and continual non-normally distributed variables were analyzed using Mann-Whitney Rank summ test. Continuous variables were compared using Student t-test.
Results: 12.532 surgical procedures were done. There were no significant differences in comparing demographic variables between studied groups. Remifentanyl mean plasma concentration in this population was 4,76 (±0,45) ng/dl for an average consumption of 0,17 (± 0,13) mcg/kg/min. being significantly high for those patients whom had undergone abdominal surgeries (p<0,025). Desflurane, sevoflurane and isorane consumptions were significantly reduced when remifentanyl was used [32 (±5), 23(±2), 15 ((±1) vs. 13 (±3); 9 (±1); 6(±1) ml/hr. (p<0,001)]. The total estimated amount of halogenated gases per month was reduced to approximately 55% (40.192 vs. 17.099 ml).
Conclusions: 1.Standardized use of remifentanyl in surgical units which use balanced inhaled anesthesia techniques, significantly reduces the usage of volatile halogenated anesthetics, and protects environment. 2.Implications derived from these observations could be useful for those institutions in various developing countries where TIVA is not feasible for economical or infrastructural reasons.
3. New associations between remifentanyl and other drugs like ketamine or midazolam, based on pharmacological interaction mechanisms could lower even more halogenated gas consumption during surgery. It is a necessity to continue searching new anesthetic technics that are friendly with our planet and at the same time giving economic benefit especially to those in poorer populations.

Differently Directed Changes in Interferon-γ Production Depending on Radioadaptive Response.

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Background: Interferon-γ (IFN-γ) is a pleiotropic cytokine with antiproliferative and immunomodulatory activities that are crucial for the regulation of immune responses.
Methods: We examined a group of military pilots. The examinees were divided into 3 subgroups: ground personnel (9 persons, control group), 17 pilots with <1000 h flight time, and 12 pilots with >1000 h flight time. The quality of reparation is in many respects genetically determined; therefore, we used peripheral blood lymphocytes from pilots for *in vitro* detection of a radioadaptive response (RAR), which was evaluated by the number of chromosome aberrations.
Results: No differences in IFN-α serum content after induction by NDV virus were detected. The adaptive response was observed in 7 individuals of the control group (78%), in 10 pilots who had <1000 flight hours (59%), and in 4 pilots having >1000 flight hours (33%). The examined individuals were divided into 2 groups depending on the presence of RAR, and IFN-γ production after radiation was measured. It was shown that at doses 0.05 Gy or 0.5 Gy no differences between groups were detected. Exposure with these doses sequentially in 48 h interval resulted to differently directed changes: lymphocytes of individuals with RAR produced more IFN-γ than before while cells of persons without RAR made it less.
Conclusions: The quality of adaptive mechanists evaluated by RAR may be useful for estimation of individual sensitivity to radiation during radiotherapy in oncology and in prediction of professional risk.

Conscious drug selection and dosing by genotyping and phenotyping of alleles with mutations, deletion and/or duplication of the CYP2D6 gene

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Background: Polymorphisms of Cytochrome p450 2D6 (CYP2D6) have a significant effect on the pharmacokinetics of most antidepressants. Individuals are classified as poor metabolizers (PMs) due to inheritance of two mutant CYP2D6 alleles and develop higher plasma drug concentrations causing an increased risk of side effects and toxicity when subjected to standard recommended doses of CYP2D6 substrate drugs. In contrast individuals are classified as ultrarapid metabolizers (UMs) due to inheritance of alleles with duplicated or multiduplicated active genes and will thus under circumstances not reach therapeutic plasma levels of CYP2D6 substrates, leading drug resistance and false accusation of non compliance. Our study aimed to develop a rapid and reliable procedure used for early detection of PMs- associated mutations and/or deletions (CYP2D6 *4, *3, *6, *9 and *5) and UMs- associated CYP2D6 gene duplication or multiplication.
Methods: EDTA blood was drawn from twenty five pre-selected depressed patients [(14 men and 11 women), mean age ±SD (49.8 ±12.7)] treated with the antidepressant venlafaxine and having unusual resulting plasma concentrations of venlafaxine were chosen for genotyping analysis. Real-time PCR reaction with subsequent fluorometric melting point analysis of the PCR product was used. Gene deletion (*5) and gene duplication or multiplication were investigated based on the measurement of the fluorescence intensity quotient (N) of the CYP2D6 gene relative to the albumin gene as an internal standard gene using a quantitative PCR technique.
Results: One homozygous (*6/*6), and three heterozygous (two with*4/*5 and one with*4/*6) were detected. Five individuals were heterozygous (*4/non detectable allele) and only one patient had heterozygote gene duplication [*4/gene duplication (2x*1)]. Six individuals had gene duplication. Melting curves were verified using DNA samples of known genotypes and by sequencing the PCR products.
Conclusions: Our new genotyping procedure was evaluated against the ratio between the O-demethylated (ODV/V) metabolite of the CYP2D6 substrate Venlafaxine (V) and determined by HPLC serving as phenotype of the genotyped patients. This genotyping procedure was regarded as fast and reliable for clinical routine.

Cardiac tamponade from slingshot metal darts in Chuuk: a retrospective review of cases.

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We determined the immediate cause of death of patients with penetrating cardiac injuries from slingshot metal darts. This retrospective review of cases focused on those 7 patients with penetrating cardiac injuries from the period July 1999 to July 2005. There were 6 patients who underwent emergency thoracotomy regardless of the type of operative approach. Five of the 6 patients who were operated underwent left Lateral Thoracotomy and 1 patient underwent Median Sternotomy. There were 11 patients who sustained cardiac injuries out of the 240 cases reviewed. The patient's with cardiac injuries had a higher mortality (27.3%) than those who have penetrating thoracic injuries (3.5%) without associated cardiac injury. Of the seven patients who had penetrating cardiac injuries, 5 patients underwent left Lateral thoracotomy and 1 patient underwent median sternotomy. All 6 patients had chest tube thoracotomy insertion prior to surgery. There were 2 deaths in this review of penetrating cardiac injuries. The other patient with 7 multiple slingshot injuries died of cardiac tamponade with hypovolemic shock.

The Long Term Follow up Results of Hydatid Cysts after Albendazole and Mebandazole Tratment

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Background: Our experiences gained over the years of study on the treatment of hydatid cyst disease showed that women are more susceptible to hydatid cyst disease then men. Anthelminthic drugs such as albendazole or mebendazole are more effective in lung cysts than the liver cysts when prescribed orally. Initially we undertook experiments to explain this phenomenon.
Methods: Cystic liver and lungs were collected freshly from the municipal slaughter house and from the patients. Each cystic fluids collected and viable scoleces were pooled and used for analysis. Scolicidal agents such as hypertonic saline solution, 0.5% silver nitrate, 25% ethanol, and 10% albendazole suspension were used at a time to determine the effect of scolicidal agents kinetically on the viability of scoleces from minutes to 72 hours assessed by eosin Y dye solution exclusion and inclusion.
Clinical use of in vitro findings: Initially, sheeps having hydatid cyst disease were selected by ultrasonic examination of the liver and lung of the animals brought to slaughter house. The animals were grouped for albendazole and mebandazole treatment. And each animal cysts were treated percutaneously with either 10% albendazole or mebandazole suspension solution. Based on sheep experiment the patients treated with saline solution by means of PAIR-PD (percutaneous puncture, aspiration, injection and irrigation, and reaspiration) and albendazole by PAI (percutaneous aspiration and injection) procedure.
Results: Among them hypertonic saline solution, ethanol, and silver nitrate treatment exerted immediate effect within half an hour while albendazole showed its effect after 48 hours. Because of these findings we used pre and post percutaneous oral albendazole or mebandazole treatment for 72 hours prior to PAIR-PD or PAI involvement. Thus, turgid cyst becomes flaccid and enables puncture without spilling the cystic content which prevents complications, dissemination and recurrence. Percutaneous drainage and irrigation application showed that protoscoleces metabolize and convert these drugs into active form to affect their viability irreversibly. Generally, patients are referred to hospital with already existing cysts. PAI of the cysts with antihelmintic drugs or PAIR-PD of scolicidal agents or saline solution cure the cyst with a minimum morbidity. The kinetic in vitro studies showed that sclerosing agents, such as ethyl alcohol, silver nitrate, saline solution kill the protoscoleces immediately and albendazole and mebandozole suspension solution starts affecting 48 hours later and kills the protoscolecess completely after 72 hours. However, sclerosing agents can cause chemical sclerosing cholangitis which limits their use.
Conclusion: The procedure described above offerred safe alternative to surgical treatment of hydatid cysts. Over 10 years follow up our procedure did not experience any mortality which is higher with surgical procedure. Complications such as anaphylactic reaction, side effects and recurrence are minimal. Hospital stay is reduced considerably and the patient can return the work the following day. Hospital cost is reduced considerably. However, the best therapy is to prevent cyst formation from the protoscoleces at early phase of infection or to prevent cyst growth by preventing the differentiation of germinative membrane. Therefore, it looks plausible to develop vaccination targeted to germinative membrane differentiation which circumvents immune system in the infected patients.

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Efficacy of amoxicillin/clavulanic acid in preventing infectious and inflammatory complications following impacted mandibular third molar extraction.

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Background: The aim of this clinical trial was to evaluate the efficacy of amoxicillin/clavulanic acid 500/125 in the reduction of infectious and inflammatory complications after extraction of an impacted mandibular third molar. Our hypothesis is there are more infectious and inflammatory complications in patients treated with placebo than in those treated with antibiotic, with a maximum ratio difference of 0.067.
Methods: A double-blind placebo-controlled randomized clinical trial. The sample was derived from the population of subjects attending Cruces Hospital for evaluation and extraction of a impacted mandibular third molar. A maxillofacial surgeon performed the operations under local anesthesia. The surgical technique was the same in all cases, and the follow-up period was 8 weeks. Patients were treated with postoperative placebo or amoxicillin/clavulanic acid 500/125 mg 3 times a day during 4 days. The outcome variable was infectious and inflammatory complications. Sex, age, smoking, molar depth, angulation, need for sectioning, ostectomy, and operation time were recorded. Analysis was by intention to treat.
Results: A total of 490 lower third molars were surgically removed (259 antibiotic and 231 placebo), The patients' mean age was 24,15 (23,70-24,59), the frequency of postoperative infectious and inflammatory complications was 1.9% in the antibiotic and 12.9% in the placebo group (OR 7.6, 95%CI 2.9-19.9; P < .001). The number needed to treat was 10 (7-16). Unadjusted relative risk was 0.15 (0.06-0.38) (P < .001). Absolute reduction risk was 0.11(0.066-0.155)]. Therefore, the hypothesis cannot be rejected. In third molars submucous (p=0.091 and NNT 17 with IC95% 8-infinite). Multivariate analysis shows treatment with antibiotic (OR = 8.66 (3.17-23.67); P < .001) and age (OR = 1.08 (1.00-1.16); P = .029) are the only variables to be included in the logistic regression model. Possibility of infection: $P(x)=1/1+e^{-(-3,74+0,074\ EDAD-2,075\ ANTIBIOTIC(1))}$ Severe complications occurred in one patient in placebo group
Conclusions: Amoxicillin/clavulanic acid is efficacious in reducing the incidence of postoperative infectious and inflammatory complications following third molar extraction but should not be prescribed in all cases. Preventive antibiotic treatment would not be indicated for third molars submucous. Age should be taken into account, infection risk increases 8% every year in all patients.

Manganese (Mn) Transport at the Blood-Brain Barrier: Implications for Parkinson's-Like Disease

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Background: Though an essential trace element, exposure to high Mn levels has been implicated in a Parkinson's-like disease, manganism. We hypothesized that symptoms associated with these two disorders reflect perturbations in shared molecular pathways. Accordingly, studies were carried out in *C. elegans* to test the hypotheses that (1) divalent metal transporter (DMT1) is a putative Mn transporter, and (2) Mn preferentially targets dopaminergic (DAergic) neurons.
Methods: Bristol wild-type (WT) *C. elegans* N2 strain was used unless otherwise indicated and was grown at 20°C in Petri dishes on nematode growth medium inoculated with OP50 *E. coli*. For lethality assessment, live and dead worms were counted on each plate and recorded. Mn content was measured by atomic absorption spectrometry. Neurodegeneration was assessed by confocal microscopy.
Results: The *C. elegans* genome encodes three DMT1 orthologues (SMF-1, SMF-2, SMF-3). *C. elegans* deletion-mutants *smf-1(eh5)* and *smf-3(ok1035)* exhibited resistance to Mn exposure compared to WT, while *smf-2(gk1330)* was more sensitive. Corroborating these observations, after exposure, Mn content in *smf-2(gk1330)* was greater than WT, *smf-1(eh5)* and *smf-3(ok1035)*, the latter taking up the least Mn of all mutants. SMF-1::GFP and SMF-3::GFP were found co-expressed in the gut and the major epidermis hyp7, likely accounting for most Mn uptake, while SMF-2::GFP was mainly expressed in the "mc1-3" and "vpi1-6" cells. Confocal microscopy revealed that Mn specifically targeted DAergic neurons, while sparing others (GABA, glutamate, and acetylcholine). The DA transporter knock-out *dat-1(ok157)* and the DA receptor *dop-2(vs105);dop-1(vs100);dop-3(vs106)* triple mutant were hypersensitive to Mn exposure. Combined with measurements of DA content in these strains, our results established that elevated DA levels sensitize the worm to Mn toxicity.
Conclusions: (1) DMT1 in a putative blood-brain barrier Mn transporter and a target for transport inhibition into the mammalian brain under conditions of excessive Mn exposure. (2) DAergic neurons are exquisitely sensitive to Mn and extracellular DA is involved in Mn-induced neurotoxicity. (3) *C. elegans* is ideally suited for studies on genetic pathways involved in Mn toxicity and PD.

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Targeting Dexamethasone-Loaded anti-E-selectin Liposomes Prevents Glomerulonephritis Progression: The Potential of Vascular Bed-Specific Drug Delivery

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Background: Glomerulonephritis is a renal disease characterized by glomerular inflammation which is frequently treated with glucocorticoids. However, their use has limitations because of systemic side effects.
Aim: To test the hypothesis that targeted delivery of dexamethasone by immunoliposomes to glomerular endothelium decreases renal injury, while preventing systemic side effects of dexamethasone.
Methods: Glomerulonephritis was induced in C57bl/6 mice and monitored for 2 weeks. Dexamethasone-containing immunoliposomes and free dexamethasone were injected intravenously. Gene expression was quantified in renal endothelial subsets after lasermicrodissection. Disease parameters analyzed included the extent of glomerular crescent formation, albuminuria, and blood ureum nitrogen and plasma glucose levels.
Results: E-selectin was expressed selectively by glomerular endothelial cells after induction of glomerulonephritis. Consequently, accumulation of anti-E-selectin (Ab_{Esel}) liposomes was 3.6 times higher than non-targeted IgG liposomes in diseased kidney. In glomeruli dexamethasone-Ab_{Esel} liposomes co-localized with endothelial cells. Targeted delivery of dexamethasone-Ab_{Esel} liposomes reduced glomerular endothelial expression of P-selectin, E-selectin and VCAM-1 by 60 to 70%. Other renal microvasculature was not affected by targeted dexamethasone delivery and unlike administration of free dexamethasone, site selective delivery of dexamethasone-Ab_{Esel} liposomes did not increase blood glucose. Dexamethasone-Ab_{Esel} liposomes reduced albuminuria at day 7 and ameliorated renal injury at day 14 as evidenced by a reduction of blood urea nitrogen levels, decreased glomerular crescent formation, and down regulation of disease associated genes.
Conclusion: 1) E-selectin is an excellent target for selective delivery of potent anti-inflammatory drugs to glomerular endothelium. 2) Recent new application of this powerful strategy includes encapsulation of gene specific small interference RNA to knock-down genes important for disease development.

Randomized Controlled Clinical Trial of Day-Care Based and Hospitalized Management of Severe Childhood Pneumonia by Injection Ceftriaxone in Dhaka, Bangladesh

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Background: Although hospital-based treatment of severe pneumonia in children is desirable, but practical barriers often prevent children in areas with the highest rates from receiving hospital care. We have shown recently in an uncontrolled trial a success in day-care treatment of severe pneumonia in children; however, a randomized, controlled trial is recommended.

Methods: The study was conducted at the Radda Clinic, Mirpur, Dhaka and Mirpur Shishu (Paediatric) Hospital, Mirpur, Dhaka as the two primary sites for the day-care management and inpatient management, respectively. Children of either sex aged 2-59 months with severe pneumonia according to World Health Organization (WHO) criteria without associated co-morbidities were randomized to receive either day-care treatment at the Radda Clinic, or hospitalized treatment at Mirpur Shishu Hospital. Children at the clinic received day-care management with appropriate antibiotics like injection ceftriaxone, feeding and supportive care from 08:00-17:00 every day, while mothers were educated on continuation of care at home during the night. Children at the hospital received hospital care with similar antibiotics like injection ceftriaxone, feeding and supportive care for 24 hours every day rather than 9 hours at the clinic until improvement.

Results: From September 2006 to December 2007, 251 children (125 at clinic as day-care, 126 at hospital as hospital care) with severe pneumonia without associated co-morbidities were enrolled, 83 (33%) were hypoxaemic with a mean (SD) oxygen saturation of 94 (5)%, which increased to 98 (1)% on oxygen therapy. Day-care management was successful in 109 children [87% (95% CI 80% to 92%)], as opposed to the success rate of 98% (95% CI 94% to 100%) by hospital management. Fifteen [12% (95% CI 7% to 19%)] children in the day-care group had to be referred to hospital for supportive management, but none in the hospital group.

Conclusion: Most (87%) children with severe pneumonia without associated co-morbidities can be successfully managed on a day-care basis at a day-care clinic. However, few (12%) children required referral to hospital.

Transcription of Major Histocompatibility Complex Class I (K^b) and Transporter Associated with Antigen Processing 1 and 2 genes is up-regulated with age.

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Background: The transporter associated with antigen processing 1 and 2 (TAP1 and TAP2) genes belong to the ATP-binding cassette family of transporter genes. They provide peptides necessary for the assembly of MHC class I molecules by transporting these peptides into the endoplasmic reticulum. As MHC class I protein expression increases with age, we have explored the effect of age on the transcription of MHC class I (K^b), TAP1 and TAP2 genes in C57BL/6 mice.
Methods: Blood and spleen lymphocytes were isolated and from mice aging from 3 months to over 24 months. RNA was extracted and mRNA for K^b, TAP1, TAP2 were quantified using slot-blot hybridization followed by a densitometry.
Results: There is a parallel age related increase (1.5-fold) in blood lymphocyte mRNA of these genes from 3 months to 21 months. In mice over 24 months old there is a decrease in K^b and TAP1 mRNA, but an increase in TAP2 mRNA. In spleen lymphocytes an age-related increase in all three mRNA species occurs throughout life. While MHC class I and Tap genes follow about the same age related changes, MHC class I mRNA is about 50 times more abundant than either TAP1 or TAP2 mRNA.
Conclusion: Transcription of MHC class I (K^b) and peptide transporter (TAP1 and TAP2) genes is up-regulated with age. It is possible that coordinated expressions of MHC class I and TAP1 or TAP2 may predispose an animal to better antigen presentation and a longer life.

The changes in renal function after a single dose of intravenous furosemide in patients with compensated liver cirrhosis

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Background: Patients with compensated Child-A cirrhosis have sub clinical hypovolemia and diuretic treatment could result in renal impairment.
Aim: To evaluate the changes in renal functional mass as reflected by DMSA uptake after single injection of intravenous furosemide in patients with compensated liver cirrhosis.
Methods: Eighteen cirrhotic patients were divided in two groups; eight patients (group 1, age 56 ± 9.6 yrs, Gender 5M/3F, 3 alcoholic and 5 non alcoholic) were given low intravenous 40 mg furosemide and ten other patients (group 2, age 54 ± 9.9, Gender 6M/4F, 4 alcoholic and 6 non alcoholic) were given high 120 mg furosemide respectively. Renoscintigraphy with 100MBq Of Tc 99 DMSA was given intravenously before and 90 minutes after furosemide administration and SPECT imaging was determined 3 hours later. All patients were kept under low sodium diet (80mEq/d) and all diuretics were withdrawn for 3 days. 8-hours UNa exertion, Calculated and measured Creatinine clearance (CCT) were performed for all patients.
Results: Intravenous furosemide increased the mean renal DMSA uptake in 55% of patients with compensated cirrhosis and these changes persist up to three hours after injection. This increase was at the same extent in either low or high doses of furosemide. (From 12.8% ± 3.8 to 15.2% ± 2.2, $p < 0.001$ in Gr I as compared to 10.6% ± 4.6 to 13.5% ± 3.6 in Gr 2, $p < 0.001$). In 8 patients (45%, 3 pts from Gr 1 and 5 pts from Gr 2) DMSA uptake remain unchanged. The mean 8 hrs UNa excretion after intravenous furosemide was above 80 meq/l and was higher in Gr 2 as compared to Gr 1 respectively (136 ± 37 meq/l) VS 100 ± 36.6 meq/l, $P = 0.05$). Finally, basal global renal DMSA uptake was decreased in 80% of patients; 22.5 ± 7.5% (NL > 40%), as compared to normal calculated creatinine clearance (CCT 101 ± 26), and measured CCT of 87 ± 30 cc/min ($P < 0.001$).
Conclusion: A single furosemide injection increases renal functional mass as reflected by DMSA in 55% of patients with compensated cirrhosis and identify 45% of patients with reduced uptake and who could develop renal impairment under diuretics. Whether or not albumin infusion exerts beneficial effect in those patients with reduced DMSA uptake remains to be determined.

How to avoid drug—drug interactions

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Background: It may be favorable to use a combination of drugs, if the combination is well documented, to enhance the effect or to reduce adverse effects. However, in the case of patients visiting several different physicians, who are prescribing less appropriate combination of drugs due to being unaware of each other, the outcome may be negatively influenced. The polypharmacy may even result in serious adverse drug events.
Methods: In Sweden, the government has legislated and funded a nationwide mandatory database for all dispensed prescriptions. The information content is available during 15 months for prescribers and dispensing pharmacists, as well as for the registered individual. Prior to the launch of the nationwide database in Sweden, studies were performed to estimate the prevalence of potential drug—drug interactions (DDI) in a general population and to evaluate the historical change in risk over three decades.
Results: On average each individual filled 14.6 prescriptions during a 15 month study period (2003-2004). The risk of receiving a potential DDI was estimated as the cumulative incidence 0.26 overall. The relative risk for women was estimated as 1.3. For more severe potential DDIs the cumulative incidence was estimated as 0.02. The risk of receiving a potential DDI was positively correlated to age and polypharmacy. The change in risk over three decades increased for type C (relative risk RR 1.18), but decreased (RR 0.71) for the more severe type D interactions. Polypharmacy increased with more than 60% during the three decade study period. Fifteen months after launch of the new National Pharmacy Register in Sweden, the prevalence of individuals with dispensed drugs was 71% (6,424,487/9,047,752). For elderly (80-89 years) the mean number of dispensed prescriptions was 27.8 during the first 15 months.
Conclusions: The new National Pharmacy Register will provide health care professionals with a powerful tool to systematically review all prescriptions. Alert systems integrated in electronic healthcare records may be used to detect potential DDIs. To gain approval among physicians, the alerting should focus on the more severe and clinically relevant DDIs. More individual-oriented information (laboratory, genetic, allergies) may in the future be processed before prescribing of drugs, to better customize the therapy for the single individual.

Distinct Cell Cycle Proteins Control Schwann Cell Proliferation in Health and Disease

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Background: Proliferating Schwann cells, the glial cells of the peripheral nervous system, are a prominent feature during early development and after damage to peripheral nerves. Altered Schwann cell proliferation is also associated with diseases and pathological states including inherited peripheral neuropathies, peripheral nerve tumors, and peripheral neuropathies secondary to diabetes, cancer chemotherapeutic agents, or toxins. To gain more insight into the molecular processes governing Schwann cell proliferation in health and disease, we examined the Schwann cell cycle and its regulation *in vivo.*
Methods: We have examined the expression, regulation, and localization of cyclins, cyclin-dependent kinases (cdk), and cell cycle inhibitors in Schwann cells of developing and adult peripheral nerves using immunohistochemistry. In addition, we used appropriate mutant mice to examine the functional requirement for the respective cell cycle proteins in Schwann cell proliferation.
Results: Proliferating Schwann cells during development express cyclin D1 in the cytoplasm. After injury, cyclin D1 becomes localized to the nuclei of proliferating Schwann cells. Cyclin D1-deficient animals revealed that developmentally regulated proliferation is not affected by the absence of cyclin D1, whereas injury-induced proliferation is impaired. We further found that the cell cycle inhibitor p21 appears first in the cytoplasm of Schwann cells at postnatal day 7 when most cells have already ceased dividing. After nerve injury, however, p21 is localized mainly in nuclei of dedifferentiated Schwann cells. Consistently, p21-deficient Schwann cells do not undergo proper growth arrest in later phases of nerve development. In contrast, after nerve injury, nuclear p21 is required for correct cell cycle control at the peak of Schwann cell proliferation. We next investigated the requirements for cdk2, 4, and 6 during Schwann cell proliferation. We show that only cdk2 and 4 are expressed in peripheral nerves. Our data from cdk-deficient mice indicate that postnatal Schwann cell proliferation is abolished in the absence of cdk4 but not in the absence of cdk2 or 6.
Conclusions: We find that distinct components of the cell cycle machinery that regulate Schwann cell proliferation during development differ fundamentally from those activated following nerve injury or in peripheral neuropathies.

Magic Bullets for the Treatment of Oxidative Stress-Induced Neurodegenerative Disorders

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A limited capacity to self-repair and regeneration of cellular damages is the hallmark of many central and peripheral regions of our body. In the last few years, evidence has accumulated that shows major damage to much type of cells that comes from *oxidation*. The source of free radicals and oxidizing agents are part of the normal cellular machinery, however, failing to remove excess of free radicals or the oxidizing agents creates a situation called oxidative stress (OS), responsible for a devastating complications which end in cell death. In the last decade, clinical trials with various antioxidants failed mainly due to the fact these compounds do not penetrate the cell.
We developed two new series of compounds consisting of small molecular weight thiol compounds that can cross the plasma membrane into the cell as well as the blood brain barrier (BBB).
i) AD4 (N-acetyl cysteine amide) is one of the first series of compounds that was shown to very efficient in vitro and in a large number of animal models, reversing the effects caused by oxidative stress (Ref. 1-10). Among its prominent properties, it is an inhibitor of MAP Kinases such as ERK1/2, JNK, and p38 *in vitro* it scavenges ROS (Reactive Oxygen Species), regenerate GSH, chelates copper ions, and shows restoration of oxidative stress markers such as cell viability (MTT assay), protein carbonyls, nitrosylation (3-nitrotyrosine), and lipids.
ii) This novel series of low molecular weight peptides is called "Redox-Cluster" (RC). They offer a prolonged action base on unique properties of cell entry and trapping inside the cell.
These compounds were also shown to be potent inhibitors of the MAP Kinases ERK1/2, JNK, and p38 *in vitro*. They are scavengers of ROS (reactive oxygen species, and show restoration of all oxidative stress markers such as Cell viability (MTT assay), protein carbonyls, nitrosylation (3-nitrotyrosine) and lipid peroxidation (levels of HNE). In studies *in vivo* a significant improvement in oxidative markers was observed.
The two families of glutathione precursors are potentially promising "magic" bullets for the treatment of neurodegenerative disorders, as well as other degenerative and multi-system disorders, such as diabetes and asthma.

Small Lipoprotein A-I subclasses (42,000-70,000) are Promising Biomarkers in Cardiovascula Disease

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Background: The predictive value of the high density lipoprotein (HDL) cholesterol as a biomarker for cardiovascular disease is now questionable. HDL contains several subclasses of varying size, composition, and function. Aims: 1) To detect and quantitate the apolipoprotein A-I-containing lipoproteins (LpA-I) directly from fresh plasma instead of HDL subclasses. 2) To study the plasma distribution of these LpAI subclasses in normolipidemics and hyperlipidemics. 3) To follow up the changes in their distribution after a fatty meal and during pregnancy.
Methods: Fresh plasma from 90 normolipidemics and 20 hyperlipidemics was subjected to gradient polyacrylamide gel electrophoresis followed by electrotransfer onto agarose gel-containing anti-apoA-I. Similarly, plasma from 8 normolipidemic sunjects was withdrawn after fasting and 4 and 6 h after a fatty meal. Two pregnant women were followed 4.5, 8.0, and 11.5 months during pregnancy and after delivery.
Results: We detected 12 different LpAI subclasses of molecular mass ranging from 42,000 to > 354,000 with Stokes radius of 2.96 to > 5.8 nm. The percentage of the smallest subclasses $SlpAI_2$, $SlpAI_3$, and $SlpAI_4$ (50,000, 45,000, and 42,000, respectively) was low in normolipidemics (7.8, 3.4, and 2.7%) and significantly (P < 0.01, 0.05, 0.05, respectively) higher in hypelipidemics (18.9, 8.3, 5.2%, respectively). In normolipidemics, the level of $SLpAI_2$ increased significantly (P < 0.05) than the fasting level 4 h after fat ingestion, and decreased after 6 h (14.1, 20.3, 19.5 mg/dL, respectively). The percentage of $SLpAI_1$ increased during pregnancy and decreased after birth (7.8, 9.7, and 6.6%), and $SLpAI_2$ was detected (4.6%) after 8 months and disappeared after birth. A strong positive correlation was observed between the SLpAI subclasses and plasma triacylglycerols (TAGs) in all normolipidemics (r = 0.41, P < 0.01) and in males of < 25 y (r = 0.70, P < 0.01).
Conclusions: 1) Significant differences in the distribution of SLpAI subclasses in plasma were detected in normo- and hypelipidemics. 2) Their plasma level increases with the increase of plasma TAGs. 3) They may be related to the lipolysis of the TAG-rich lipoproteins. 4) The variation of $SLpAI_2$ level was more evident than the others and it may be a good candidate as a biomarker for cardiovascular disease.

Investigation of SGK-1 and Dexras1 expression in Human Embryonic Kidney (HEK 293) cells

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Adrenal corticosteroids are involved in multiple aspects of CNS function — for example, the feedback inhibition of the hypothalamic-pituitary adrenocortical axis. Typically, the action of glucocorticoids is mediated by rapid induction of mRNA and protein synthesis. Potential mediators of glucocorticoid action include dexras1, (activator of G protein signaling 1) first reported as a rapidly induced mRNA in pituitary tumour cells. The rapid induction of dexras1 in response to glucocorticoids in pituitary cells raises the possibility that it may be involved in negative feedback regulation of corticotropin secretion. In the present study we examined the induction of dexras1 in human embryonic kidney (HEK293) cells which have been used as a model for glucocorticoid-mediated regulation of corticotropin secretion.
To verify that HEK 293 cells contain functional glucocorticoid receptors (GR) activated by dexamethasone, MMTV-LTR plasmid, which responds to activated-GR by enhancing luciferase expression via the MMTV promoter, was transfected into the HEK 293 cells. 48 h after transfection, the cells were treated with dexamethasone. Analysis of the time-course of dexamethasone action on MMTV regulated luciferase activity revealed that luciferase induction was maximal at 120 min. Exposure to various concentrations of dexamethasone for 120 min produced an increase of MMTV regulated luciferase activity in HEK 293 cells in a concentration -dependent manner. Maximum effect was obtained with 100 nM dexamethasone (up to 5-fold).
The expression of dexras1 was assessed by Northern blot. The results of Northern blot showed a 5 kb dexras1 and a 2.6 kb serum and glucocorticoid-induced protein kinase (SGK-1) mRNA (a positive control for glucocorticoid induction) species in HEK 293 cells. Dexamethasone had no significant effect to amount of dexras1 mRNA induction for varying times (0-120 min) but increased SGK-1 mRNA with maximum effect at 30 min. The analysis of SGK-1 protein in HEK 293 cells also showed significant increase in response to dexamethasone.
The results suggest that; 1) HEK 293 cells respond to dexamethasone via an endogenously expressed GR; 2) Dexras1 is not a glucocorticoid-induced protein in HEK 293 cells; 3) SGK-1 is induced by dexamethasone in HEK 293 cells.

Value of pharmacokinetic/pharmacodynamic in dose management of ceftazidime and imipenem in ICUs

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Background: The purpose of our study was to assess the value of serum assay of ceftazidime (CAZ) and imipenem (IMI) in patients in the intensive care unit (ICU) of the Saint-Etienne University Teaching Hospital and in other ICUs in the region with regard to optimisation of treatment management.
Methods: Between 01/11/05 and 29/02/08, in patients hospitalised in ICUs, not on dialysis and undergoing treatment with CAZ given in a continuous infusion or with IMI (non-continuous), serum assay of the respective antibiotics were performed 36 hours after the start treatment using a single serum sample for CAZ and determination of trough and peak concentrations for IMI. Assays were performed using the microbiology technique with results 18 hours after sample collection. CAZ 2 g was given systematically in a bolus at the start of treatment.
Results: Assays were performed in 92 and 105 patients respectively for CAZ and IMI. Mean patient age was 66 years (19 to 89 years) and mean weight was 73 kg (33 to 122 kg). The dosage was between 1 g and 6 g/24 h for CAZ and between 1 g and 6 g/24 h for IMI. The mean serum CAZ concentration was 46.9 mg/L (7.4 to 162.3 mg/L). Serum CAZ concentrations were as follows: 35 to 65 mg/L in 37% of patients, < 35 mg/L in 43.2% and > 65 mg/L in 19.8%. Infection was established in 51 patients, with 42 strains of *P. aeruginosa* detected. The serum concentration / MIC ratio was >= 5 for 84.3% of patients and > 10 for 65.6% of patients. Trough concentrations of IMI were < 0.5 mg/L for 14.7% of patients, between 0.5 and 2 mg/L for 43.2%, and > 2 mg/L for 42.1%. The mean peak concentration of IMI was 19.9 mg/L (3 to 78 mg/L). Infection was recorded in 47 patients, including 34 enterobacteria and 11 *P. aeruginosa*. Antibiotic dosage was adjusted respectively for CAZ and IMI in 19.8% and 28.4% of patients based on the initial assay results.
Conclusion: Our study shows that assays are needed in ICUs to confirm the efficacy of time-dependent antibiotics (ß-lactams), to avoid treatment toxicity, to achieve efficacy as rapidly as possible and to avoid selection of resistant mutants, particularly in strains having limited susceptibility to antibiotics.

The Role of Endothelial Cell Heterogeneity in the Search for Anti-angiogenic Agents

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Background: In the late 1800s, Paul Ehrlich documented the clear differences among endothelial cells from different organs by describing the uniqueness of the brain-associated vasculature, the blood-brain barrier. Nevertheless, endothelial cell heterogeneity, aptly demonstrated by the blood-brain barrier, was not generally appreciated until the last decade and even now has not become a major consideration in developing angiogenic and anti-angiogenic therapies. Recognizing the importance of this heterogeneity is critical for the development of "*magic bullets*" against cancer or immune-mediated diseases, inasmuch as there is no single uniform target in the vasculature.
Methods: There are multiple methods for studying angiogenic and anti-angiogenic agents, ranging from *in vitro* models to various *in vivo* assays. Critical to the choice of targets is the recognition of endothelial cell heterogeneity. We and others have developed numerous endothelial cell lines from various microvascular organ beds, from large arteries, from specific veins, and from tumors.
Results: Responses to different agents *in vitro* varies with the source of endothelial cells used in the assays. *In vivo* results depend on the site of injection, the physical topography, and the organ environment. The vasculature of developing embryos differs from that of adults, and there are critical distinctions between vessels of the lymphatic system and those of the blood vasculature. Examples of both *in vitro* and *in vivo* studies of effects of various pharmacological agents will be presented.
Conclusions: Endothelial cell heterogeneity presents an important challenge to the development of pharmacological reagents ("*Magic Bullets*") when attempting to target the vasculature of specific organs, different tumors, or selected sites of inflammation. Paul Ehrlich pioneered in an area of pathology whose importance is only now beginning to be appreciated.
Dedication: This contribution is presented in honor of Dr. Judah Folkman, long-time colleague and friend you died unexpectedly earlier this year.

Targeted delivery of cisplatin using polymeric nanoparticles

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Background: Cisplatin use is associated with serious side effects. The more selective delivery (targeting) of cisplatin to tumor cells would reduce drug toxicity, improving its therapeutic index. Aim: To develop long-circulating cisplatin-loaded nanoparticles and test their anticancer activity in mice cancer models.
Methods: A) Nanoparticles preparation: Cisplatin-loaded poly(lactide-co-glycolide)-polyethyleneglycol (PLGA-PEG) nanoparticles were prepared by a double emulsion method. B) Determination of cisplatin profiles in blood circulation: BALB/c mice were administered intravenously with PLGA-PEG/cisplatin nanoparticles and at predetermined time intervals blood samples were obtained and assayed for platinum. C) Evaluation of the tolerance of BALB/c mice to the PLGA-PEG/cisplatin nanoparticles: 5 mice groups (n=3) received 3 intravenous injections at 7-day intervals. Three groups of mice received PLGA-PEG/cisplatin nanoparticles with a cisplatin content of 2, 5 or 10 mg cisplatin/Kg. The fourth group of mice received blank nanoparticles and the fifth group of mice (n=3) received 100 µl saline. D) Evaluation of anticancer activity of PLGA-PEG/cisplatin nanoparticles: HT 29 tumor cells were injected sub-cutaneously into the left flank of SCID mice. Fifteen days later, the mice (n= 6-8) were injected intravenously 5 times at weekly intervals with free cisplatin or cisplatin-loaded nanoparticles at the same dose (5 mg/kg on a cisplatin basis).
Results: The entrapment of cisplatin in the nanoparticles resulted in a significant prolongation of cisplatin presence in blood. Balb/c mice tolerated 3 weekly intravenous injections of a relatively high dose of blank PLGA-mPEG nanoparticles (500 mg/Kg) and 3 weekly intravenous injections of a high dose of nanoparticle-entrapped cisplatin (10 mg/Kg). The cisplatin-loaded PLGA-mPEG nanoparticles was effective at delaying tumor growth in HT 29 tumor-bearing SCID mice. The group of mice treated with cisplatin-loaded nanoparticles exhibited higher survival rate compared to the free cisplatin group
Conclusions: 1) PLGA-mPEG/cisplatin nanoparticles could prolong cisplatin residence in blood and they were well tolerated by normal Balb/c mice even when relatively high doses were administered to mice. 2) The PLGA-mPEG/cisplatin nanoparticles reduced tumor growth in SCID mice with HT29 xenografts, and these mice exhibited higher survival rate than free cisplatin.

The Effect of Melatonin and Zinc on the Immune Response in Experimental Toxoplasma Retinochoroiditis

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Background: Toxoplasmosis is a zoonotic disease and the most common cause of infection of the human retina . In humans and animals, the primary control mechanism of *Toxoplasma gondii* (T. gondii) infection involves T lymphocytes. One of the potential beneficial approaches to improve the immune defense against T. gondii infestation is zinc supplementation, since due to its catalytic and regulatory functions; zinc can enhance resistance to infections. Other potential approaches that deserve further investigation in this respect are the effect of melatonin (MEL) deficiency and artificial MEL supplementation on the immune response to T. gondii infection. MEL is a hormone secreted by pineal gland that has both direct and indirect effects on the immune system. In the current study, we investigated the impact of MEL and/or zinc deficiencies and artificial MEL and/or zinc supplementations on immune and inflammatory responses in the rat model of toxoplasma retinochoroiditis.
Methods: Eighty-four Sprague Dawley male rats were divided into 12 equal groups. All groups, except controls were infected with T. gondii parasite by intraperitoneal injection. Combinations of zinc deficient diet, pinealectomy (Px), artificial zinc, and MEL were supplied during a 1 month period. At the end of the experiment, retinal and choroidal total lymphocytes, CD3+, CD4+, and CD8+ cell numbers were counted in histological sections.
Results: The highest amount of cellular infiltration (lymphocytes, CD3+, CD4+, CD8+ cells) in the choroid and retina were detected in infected+MEL+zinc treated rats and the least amount of cellular infiltration was observed in Px+zinc deficient diet treated rats. Although single zinc or MEL supplementation had no significant impact on the cellular infiltration in the retina and choroid in Px rats, combined therapy significantly improved these responses.
Conclusion: Artificial supplementation of MEL and zinc should be considered as an adjunctive therapy to classic treatment of Toxoplasma retinochoroiditis especially in immunosuppressed and elderly patients if our data will be confirmed in a clinical setting.

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Preventing Immune Evasion as a Strategy for Enhancing the Effectiveness of Herpes Simplex Virus Vaccine.

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Background: Herpes simplex virus-1 (HSV-1) and HSV-2 glycoprotein C (gC) are immune evasion proteins that inhibit complement activation by binding complement component C3b. We previously reported that mice passively immunized with a gC1 monoclonal antibody (MAb) were protected from HSV-1 challenge only if the MAb blocked the interaction between gC1 and C3b. We also demonstrated that immunizing mice with gC1 protein induced antibodies that blocked C3b binding and protected mice against HSV-1 challenge by blocking immune evasion.
Method: We use the mouse flank (epidermal disease) model to evaluate whether immunizing with gC1 protein increases the efficacy of a glycoprotein D (gD1) subunit vaccine when challenged with HSV-1. We first defined a gD1 immunizing dose that produced partial protection against HSV-1 challenge. Our rationale for partial protection was that the gD2 subunit vaccine used in human trials provided only limited protection, and we wanted to reproduce these results in mice. We then immunized mice with either gD alone or with both gD and gC and challenged with HSV-1.
Results: We found that when gC1 was added to gD1 immunizations, mice were significantly protected from epidermal disease compared with gD alone. Importantly, the combined immunizations were more effective than gD alone in preventing infection of dorsal root ganglia. Passive immunization of anti-gC1 IgG obtained from mice immunized with gC1 protein protected complement intact mice, but not C3 knockout mice.
Conclusion: We conclude that immunizing with gC1 blocks immune evasion and enhances the efficacy of a gD1 subunit vaccine.

Levetiracetam in the Treatment of Neuropathic Pain: Evidence from Cellular and Behavioral Pain Models

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Background: Neuropathic pain is a common, complex, costly pain condition, "initiated or caused by a primary lesion or dysfunction in the nervous system", presenting a major healthcare and social problem. Despite an increasing number of trials there is currently no satisfactorily effective therapy for neuropathic pain. Similarities between the pathophysiological phenomena observed in some epilepsy models and in neuropathic pain models justify the use of anticonvulsants in the symptomatic management of neuropathic pain. We attempted to elucidate the effectiveness of levetiracetam in neuropathic pain state in mice model and subsequently cellular mechanisms involved in a cellular nociceptive model.
Methods: The in vivo nociceptive behavioural "hot-plate test" was performed in normal and diabetes (streptozocin 200 mg/kg i.p.)-induced adult male Balb/C mice.
Subsequent to behavioral testing, electrophysiological measurements and calcium imaging experiments were performed on cultured neurones of the rat dorsal root ganglia (DRG) using the whole cell patch-clamp technique and the fura-2 ratiometric fluorescence microscopy. Data were analyzed using Kruskal-Wallis One-way Analysis of Variance (ANOVA), Dunnett's and Students' t tests, where appropriate.
Results: While levetiracetam had (60-900 mg/kg) no significant effect on the nociceptive threshold in normal mouse much lower doses (≤200 mg/kg) significantly restored the pain threshold latency in diabetic mice, in a dose-dependent manner. Current clamp recordings from DRG cells indicated that levetiracetam caused membrane hyperpolarisation and reduction of multiple action potential firing. Estimation of reversal potentials of levetiracetam-induced hyperpolarizing currents indicated involvement of K^+ channels. Furthermore, levetiracetam dose-dependently suppressed the depolarisation (high KCl) - induced intracellular calcium signals in DRG neurons.
Conclusions: Results obtained from in vivo behavioral tests and cellular electrophysiological and ratiometric fluorescence measurements in rat sensory neurons with agreement lend support to the validation of the promising therapeutic potential of the new anticonvulsant levetiracetam for the management of neuropathic pain.

Insulin, IGF-1 and Rosiglitazone: How Do They Effect The Glucose Metabolism and Insulin Resistance in Human SH-SY5Y Cells with Alzheimer Key Proteins?

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Background: IGF are potent neurotrophic and neuroprotective factors that reverse the effects of Ab25–35-, 1–40- and amylin induced neurotoxicity in hippocampal cells. IGF-I protects hippocampal neurons against Ab-induced toxicity. Furthermore, insulin resistance leads to a functional decrease in insulin receptor (IR)-mediated signal transduction in the brain, again consistent with the hypothesis that hyperinsulinemia or insulin resistance may potentiate the risk of AD. In this study, the effects of insulin, IGF-1 and rosiglitazone on normal and transfected SH-SY5Y cells (P301L and hTau40) with key proteins involved in Alzheimer's disease (AD) have been investigated.
Methods: The cell lines were cultured in different glucose mediums (0-100mM) to identify the effects of insulin (5µg/ml), IGF-1 (1-10-100ng/ml) and rosiglitazone (20µM) in altered glucose concentrations. ATP synthesis and the mitochondrial membrane potential (MMP,ΔΨm) were measured.
Results: Insulin was found to increase the ATP and the MMP significantly in SH-SY5Y cells in all glucose levels. However, insulin didn't cause any changes in low glucose medium in P301L cells whereas it increased these values in high glucose environment ($p<0.05$). In hTau40 cells insulin increased the ATP synthesis in low glucose medium whereas the ATP synthesis reduced in these cells in high glucose medium ($p<0.05$). Rosiglitazone slightly lowered the ATP sythesis and the MMP in normal cells in low glucose medium, and slightly increased in high glucose medium. In hTau40 ($p<0.05$) and P301L ($p<0.001$) cells rosiglitazone alone decreased the ATP synthesis whereas the cells were prevented significantly from this reduction by the combination of insulin and rosiglitazone ($p<0.05$) which reveals that there is a probable insulin resistance in transfected cells where the ATP synthesis may be promoted by rosiglitazone in the presence of insulin. 100ng/ml IGF-1, increased the ATP levels in low glucose medium and this increase was greater then the insulin had in normal SH-SY5Y Neuroblastoma Cells. 1 and 100ng/ml of IGF-1 had slightly lowered the ATP levels in high glucose level medium whereas 10ng/ml of IGF-1 had no significant effect on the ATP synthesis in SH-SY5Y cells. In P301L cells, in the 0 and 25mM glucose medium 100ng/ml IGF-1 lowered the ATP synthesis significantly. In the hTau40 cells there were not any significant effect of IGF-1 in ATP synthesis. When we compare the MMP, all concentrations of IGF-1 had lowered the MMP in 0 and 100 mM glucose mediums but increased the MMP especially in 25mM glucose ($p<0.05$) in normal SH-SY5Y Neuroblastoma Cells. However, it didn't have any effects on P301L cells. IGF-1 only lowered the MMP in hTau40 cells in high glucose (100mM) mediums.
Conclusion: Since brain possesses high energetic requirements, any decline in brain mitochondria electron chain could have a severe impact on brain function and particularly on the etiology of neurodegenerative diseases. Therefore, to identify the relationships between insulin, IGF-1 and rosiglitazone combination could be beneficial to find out novel treatment alternatives for the insulin resistance in late on-set of AD.

Antimicrobial Resistance by *Mycoplasma species* in Farm Animals.

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Background: More than 125 *Mycoplasma species* have been identified, many being pathogens in farmed animals. Mycoplasmas lack a cell wall, hence they are not susceptible to penicillins and some other antimicrobials. Mycoplasma infections are usually treated with tetracyclines, macrolides, chloramphenicols, aminocyclitols and the fluoroquinolones, however antimicrobial resistance to all of these has been reported. The treatment of mycoplasma infections is rarely successful and often requires repeated treatments. Although *Mycoplasma species* are not usually zoonotic, this repeated use of antimicrobials and development of resistance may have implications for the critically important antimicrobials used in human health. MIC data from UK bovine and ovine mycoplasma isolates and resistance mechanisms in *M. bovis* is presented.
Methods: *In vitro* microbroth dilution minimum inhibition concentrations (MIC's) were determined for 14 antimicrobials against *M. bovis* and *M. ovipneumoniae* isolates. Sensitive *M. bovis* isolates were subcultured at sub-MIC levels in 14 different antimicrobials to induce antimicrobial resistance *in vitro*. Where antimicrobial resistance had been induced this variant DNA was used to PCR the domains II and V of the 23S rRNA gene and the *gyr*A and par*C* genes, which were then sequenced.
Results: Isolates of *M. bovis* and *M. ovipneumoniae* gave high *in vitro* MIC values against most classes of antimicrobials, except the fluoroquinolones, although high fluoroquinolone MIC's have since been recorded in field isolates. The rate of development of antimicrobial resistance varied between isolates and between antimicrobials, but resistance was induced by all isolates to all antimicrobials. Fluoroquinolone resistant isolates showed a single base change (G to A) at position 259 and (C to T) at position 248 in the *gyr*A gene, observations that have been shown to confer fluoroquinolone resistance in *E. coli.*
Conclusions: It is unlikely that the genetic exchange of resistance genes occurs between *Mycoplasma species* and zoonotic organisms, however the treatment of *Mycoplasma* infections in farmed animals requires careful selection and monitoring of antimicrobial use to maximise the effectiveness of treatment and to evade the further development of antimicrobial resistance.

Cell Hydration as a Universal and Extra-Sensitive Biomarker for Determination of the Functional State of the Organism

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Background: Although the functional significance of intracellular water is widely accepted, its messenger role in signal transduction and generation of different diseases is not adequately studied by the researchers. My presentation contains a review of our data on the metabolic regulation of cell hydration and its physiological significance in norm and pathology.
Methods: Neuronal, muscle and reproductive cells, isolated tissue and organs of animals and woman breast cancer tissues serves as a subject for investigations. The light microscopic, whole cell and patch clamp, isotope, standard biochemical and genetic engineering methods were used.
Results: The data showed a close correlation between cell hydration and number of functionally active membrane proteins, having enzymatic, chemoreceptive and ionoforetic properties. The data on the Table show that the number of ouabain receptors (Na/K pump units) depends on membrane surface, which changes upon the effect of factors causing the increase of membrane permeability. The correlation between the Na/K pump regulating the cell hydration and intracellular signaling system was also shown. It makes the cell hydration as a universal and extrasensitive cell marker determining the cell functional state and a sensor for different extraweak environmental signals. By specific mRNA-induced expression in oocites membrane was shown that the cyclic nucleotide–dependent Na/Ca exchanger plays a crucial role in cell volume regulation, when the Na/K pump is inactive (cell pathology).
Conclusions: 1) The number of functionally active protein molecules in cell membrane depends on cell active surface. 2) There are a negative feedback between Na/K pump and cell hydration and a positive feedback between membrane permeability and cell hydration. 3) The cell overhydration is a marker for cell pathology. 5) The Na/K pump regulating the cell hydration is a universal and extrasensitive sensor for various environmental factors.

[^{3}H] Ouabain concentration	Incubation medium				
	Hypotonic	Isotonic	Hypertonic	Ach 10^{-4} M	GABA 10^{-4} M
$1x10^{-9}$	32.0±2.2	21.1±1.4	12.2±0.9	30.54±1.55	27.63±3.17
$1x10^{-8}$	793±45.6	508±30.1	283±19.4	270.93±28.53	174.48±13.54

TABLE: Binding of [^{3}H] Ouabain (x10^8 molecules/mg dry weight) to *Helix* Pomatia Cell Membrane in different mediums

Effects of Alcohol and Sucrose Intake on Rat Liver Cyp2e1

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Background: Ethanol metabolism by CYP2E1 leads to a significant reactive oxygen species (ROS) release, accompanied by the defense systems decrease against oxidative stress. Since expression of CYP2E1 is very much influenced by nutritional factors, specially carbohydrate consumption, and various results concerning the expression of CYP2E1 were obtained with low-carbohydrate alcohol liquid diet or the intragastric tube feeding model that also utilizes a low carbohydrate diet, this study describes the effects of ethanol and sucrose treatment on CYP2E1 levels using an *ad lib* model.
Methods: Male Sprague-Dawley rats were fed *ad. lib.* for 1, 2, 3 or 4 weeks a commercial diet (Purina Ind., Brazil) plus a 25% ethanol-20% sucrose solution. Control groups were isocalorically pair-feed to the leading ethanol-consuming animals, or received isocaloric amounts of sucrose for pairing only ethanol calories. Eighteen hours before sacrifice ethanol was withdrawal and animals had only free access to tap water or they were offered food and water *ad. lib.*
Results: Our results have shown that ethanol administration was associated with CYP2E1 induction, otherwise CYP2E1 stabilization was more associated to sucrose consumption.
Conclusions: Our findings indicate that dietary deficiencies, especially low carbohydrate intake could be crucial in the CYP2E1 stabilization.

EM703 a New Derivative of Erythromycin, Inhibits Lung Fibrosis Induced by TGF-β Signaling in Murine and Human Lung Fibroblasts

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Background: 14-membered ring macrolides have been effective in chronic airway inflammation and also prevented lung injury and fibrosis in bleomycin -challenged mice via anti-inflammatory effects. EM703 is a new compound of Erythromycin (EM) without bactericidal effects. We investigated the anti-inflammatory and antifibrotic effects of EM703 in 1) experimental murine fibrosis model induced by bleomycin and 2) murine and human lung fibroblast cell lines.
Methods: 1) Seven weeks old male ICR mice (eight mice/group) were used. Bleomycin was administered intravenously to mice at day 0. EM703 was orally administered daily to mice. All groups were examined for cell populations in bronchoalveolar lavage fluid, and for induction of mRNA of Smad3 and Smad4 in lung tissues by RT-PCR at day 7. Fibroblastic foci were assessed histologically and hydroxyproline content was chemically determined in lung tissues at day 28.
2) We also assessed proliferation and soluble collagen production, and examined induction of mRNA of smad3 and smad4 by RT-PCR in lung fibroblast cell line MLg2908. We examined smad3, smad4, smad7 and phosphorylated smad2/3 (P-smad2/3) protein assay by western blotting in lung fibroblast cell lines.
Results: Bleomycin-induced lung fibrosis, infiltration of macrophages and neutrophils into the airspace were inhibited by EM703. Expression of smad3 and smad4 mRNA was clearly attenuated by bleomycin, but recovered by EM703. EM703 also inhibited fibroblast proliferation and the collagen production in lung fibroblasts induced by TGF–β. Expression of smad3 and smad4 mRNA in murine lung fibroblasts disappeared by TGF–β, but recovered by EM703. EM703 inhibited expression of phosphorylated-smad2/3 and smad4 protein in murine lung fibroblasts induced by TGF–β.
In human lung fibroblast, EM703 inhibited the augmentation of Smad3 mRNA induced by TGF–β. Inhibited Smad3 mRNA by TGF–β was augmented by co-incubation with EM703.
Conclusions: These findings suggest that EM703 improves bleomycin-induced pulmonary fibrosis in mice by actions of anti inflammation and regulation of TGF–β signaling, which is associated with inhibition of phosphorylation of Smad2,3 through recovery of Smad7 level.

Significant Interactions between some Antibiotics and Antimalarial Drugs

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Background: Coadministration of antibacterial and antimalarials is common in the tropics as a result of frequent association of malaria with other infections. The combination of ampicillin and cloxacillin (AM-CL) prescribed in many infections produces a broad-spectrum antibiotic activity against both Gram-positive and Gram-negative bacteria. The current studies investigated effects of chloroquine(CQ), proguanil(PG), quinine(QN) and artesunate(ART) on the bioavailability and activity of cloxacillin(Clox) and ampicillin(Amp) in vivo and in vitro.
Methods: 7 healthy volunteers received single oral doses of Clox alone followed by Clox + PG, another 8 volunteers received AM-CL alone and AM-CL+ CQ, while another 14 volunteers received AM-CL alone and AM-CL + ART in a cross-over manner. Total urine voided was collected at various time intervals. Clox in urine was determined by HPLC. Effect of CQ and PG on dissolution of Amp and Clox and on their antibacterial activity against *E. coli* and *S. aureus* was investigated.
Results: CQ showed a significant decrease in total amount (Du∞) and % dose of Clox excreted in urine by 64%, while PG led to 48% decrease. Ongoing study reveals 90% reduction of Clox by QN. In vitro dissolution revealed >40% reduction in % Amp and Clox dissolved in the presence of CQ. Similar results were obtained with PG. The MIC of Amp was increased two- to four-fold from 5.42 µg/ml to 10.83 & 21.66 µg/ml by CQ, an indication of reduction in bactericidal activity of Amp. Similar results were obtained between Clox and CQ. 9 out of 14 subjects (64%) showed 39% decrease in urinary excretion of Clox while only 5 (36%) showed an increase of 27% when AM-CL were coadministered with ART.
Conclusion: These results indicate significant drug-drug interactions between AM-CL and antimalarials in a way that correlates in vitro with in vivo findings. The urinary pharmacokinetic studies indicate marked reduction in bioavailability of Clox when coadministered with CQ, PG and QN and ART. The in vivo interactions may be due to interference with the dissolution of Amp and Clox in the body by the antimalarials. Though clinical implications of the findings are inconclusive, there should be caution in prescribing these classes of drugs together to avoid sub-therapeutic levels, which can lead to treatment failure and drug resistance.

Toxins and Adhesion Factors Associated with *Staphylococcus Aureus* Isolated from Urinary Tracts Infections

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Background: *Staphylococcus aureus* infections are widely prevalent in West Africa and are often associated with urinary tract infections (UTIs). Virulence factors from *S. aureus* have rarely been described for such infections. The purpose of the current study was to determine the prevalence of toxins and adhesion factors obtained from *S. aureus* isolated from presumed primary UTIs at the Cotonou University Hospital (CUH) in Benin as compared with the Strasbourg University Hospital (SUH) in France.
Methods: Both ambulatory and hospitalised patients were included in the study. Sixty-five independent strains of *S. aureus* from CUH and 35 strains from SUH were obtained over a four-month period. Virulence factors were characterised by immunodetection or multiplex polymerase chain reaction, and meticillin susceptibility was recorded. Approximately 50% of all isolates produced at least one enterotoxin.
Results: No isolate from SUH produced PantoneValentine leucocidin (PVL), whereas 21.5% of the *S. aureus* isolates from CUH produced PVL ($P < 0.01$). Six of 14 (43%) PVL positive isolates were meticillin-resistant. At SUH, the incidence of MRSA (57%) was significantly higher ($P < 0.01$) than at CUH (14%). Genes encoding clumping factor B, and elastin and laminin binding proteins were detected in almost all isolates (80%), irrespective of the geographical origin.
Conclusions: The results for elastin binding protein differed significantly from published data regarding isolates from other clinical origins. Staphylococcal toxins and adhesion factors may be important in the physiopathology of UTI.

Panton-Valentine leucocidin as a major virulence factor associated to furuncles

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Secretion of the Panton-Valentine leucocidin by *S. aureus* is associated to furuncles in more than 90% of isolates and in cases of furuncles. Besides, it also is associated with community pneumonia. However, most investigations considered only limited series of virulence factors being produced by these isolates.
To determine whether Panton-Valentine Leucocidin is an essential factor associated, we determined production of toxins or the presence of genes encoding enterotoxins, leucotoxins, epidermolysins, Epidermal Differentiation Inhibitor (EDIN) factors, and 8 adhesion factors for 16 isolates from VIH^- patients with furuncles, 9 isolates from VIH^+ patients, and 30 isolates from secondary skin infections, all independent isolates from the Cayenne's hospital (French Guiana). Antibiotic resistant was also performed.
Only one of the isolates from furuncles do not produce Panton-Valentine leucocidin, while only three isolates from the secondary skin infections produce this toxin. The difference between these incidences is statistically significant (Student *T* test, $p<0.001$). Concurrently, 14/30 isolates from secondary skin infections and 12/25 isolates from furuncles produced at least one enterotoxin. One isolate amongst all produces Toxic Shock Syndrome Toxin-1, 5/30 isolates from secondary skin infections produce epidermolysin A, and 1/25 from furuncles produces epidermolysin B. No isolate carries any gene encoding Epidermal differentiation Inhibitor factors A, B, or C. Concerning adhesion factors, neither the presence of genes encoding clumping factor B, collagen binding protein, bone-sialoprotein, laminin binding protein, elastin-binding protein, fibronectin and fibrinogen binding proteins significantly differs in each group of isolates. In addition, there was no significant statistical difference for the occurrence of virulence for isolates from VIH- or VIH+ patients.
Therefore, Panton-Valentine leucocidin can be presumed as the most essential causative factor for furuncles, and immune disorders as acquired immunodeficiency does not seem to contribute to any susceptibility in developing furuncles.

Transdermal Drug Delivery into and Beneath the Skin - Application to Anti Inflammatory Drugs

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Background: α-Melanocyte-stimulating hormone (α-MSH) is an endogenous neuro-immunomodulatory peptide that has potent anti-inflammatory effects. We hypothesize that this compound can be developed as a topical formulation for the therapy of psoriasis and contact dermatitis. The stability of α-MSH, and *in vitro* and *in vivo* percutaneous absorption from various dermatological vehicles was investigated in rats. The anti-inflammatory effect of topically delivered α-MSH was tested on an allergic contact dermatitis (ACD) mouse model.
Methods: The stability of α-MSH in ethanol-water (1:9) at various temperatures (40 to 70°C) and pH conditions (pH 1.0 to 10.0) was examined. The *in vitro* permeation and skin retention kinetics of α-MSH as a saturated solution in various dermatological vehicles were studied using hairless rat skin and Franz diffusion cells. The *in vivo* skin penetration of α-MSH was studied in rats by a dermal microdialysis technique. The anti-inflammatory effect of selected topical formulations (0.25 and 0.5% of α-MSH in 10% n-methyl-2-pyrrolidone and 50% ethanol) was evaluated in the ACD mouse model.
Results: Stability studies indicated that α-MSH possess an ~110 day shelf-life (time of 10% degradation) as determined by HPLC. Further, α-MSH demonstrated good stability in the pH region between 3.0 and 8.0. Permeation studies indicated that ethanol, transcutol and propylene glycol (PG) and ethanol vehicles had maximum permeation of α-MSH through the skin (between 5.0 and 7.5 µg / 24 h). Ethanol demonstrated the maximum skin retention (2.0 µg /mg) as compared to all other vehicles for which the skin retention was < 1.0 µg /mg. Dermal microdialysis results show the ethanol formulation produced a maximum concentration (C_{max}) in dermis of 6.467±2.11 ng/ml as compared with the PG formulation with a C_{max} of 2.565±1.284 ng/ml, demonstrating 2.5 fold higher dermal levels by the ethanol formulation. α-MSH formulations demonstrated significant anti-inflammatory activity in an ACD mouse model. The data indicate that like dexamethasone, α-MSH was effective in reducing the ACD response.
Conclusions: Stable α-MSH can be formulated for effective topical delivery into skin layers to demonstrate significant anti-inflammatory activity in an ACD mouse model.

Is GRP78/BiP, a Master-Regulator of Defensive Unfolded Protein Response, a New "Magic Target"? GRP78/BiP-targeting Cytotoxin and ER Stress-Inducing Drugs Synergize to Kill Cancer Cells

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Background: Diverse insults increase the amount of unfolded/misfolded proteins in the endoplasmic reticulum (ER). This leads to activation of a defensive unfolded protein response, which is controlled by ER-resident protein, GRP78/BiP. Whether the cell dies or survives the ER-stress is determined by the amount of available GRP78/BiP. Recent discovery that GRP78/BiP is the only known substrate for the proteolytic A subunit (SubA) of a novel bacterial AB_5 toxin provides the first opportunity for targeted destruction of GRP78/BiP. Aims: 1) To establish if targeted delivery of SubA into tumor cells is selectively cytotoxic. 2) To establish if destruction of GRP78/BiP leads to enhanced toxicity of ER-stress inducing drugs.
Methods: SubA was genetically fused to human epidermal growth factor (EGF) and the resulting EGF-SubA was expressed in E.coli. Tumor cells (MCF7, PC3, F98, F98-EGFR, MDA231luc) were treated with EGF-SubA alone or in combination with various drugs. Biochemical and cell biology methods were used to characterize cellular EGFR levels, GRP78/BiP cleavage, activation of unfolded protein response, apoptosis, and cell viability.
Results: Exposure of cells expressing high levels of EGFR to EGF-SubA results in rapid (~2 hours) EGFR-mediated destruction of GRP78/BiP. Despite the ongoing cleavage, in most cells it results in significant upregulation of GRP78/BiP level by 24h of treatment. EGF-SubA is highly cytotoxic to growing and confluent cells with high level of EGFR (> 10^5 EGFR per cell), with IC_{50} in the range of 3 to 40 pM, while EGFR-negative cells are at least 500-fold less sensitive. EGF-SubA strongly synergizes with thapsigargin, an ER-stress inducing drug, with ~10-fold enhanced efficacy of the drug combination, relative to each compound alone. Less prominent synergism is observed with drugs that are less stressful for ER.
Conclusions: GRP78/BiP might be a new "Magic Target". Its targeted destruction with subnanomolar concentrations of EGF-SubA is extremely cytotoxic, while non-toxic concentrations of EGF-SubA disarm cellular defense and allow to use virtually non-toxic amounts of ER-stress inducing drugs.

The Strong Growth Advantage in Stationary Phase Phenomenon in Mixed Cultures of Antimicrobial Resistant *Escherichia* and *Salmonella*

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Background: During prolonged stationary phase mutants with increased fitness express **g**rowth **a**dvantage in **s**tationary **p**hase" (GASP) enabling them to grow and displace the parent as the majority population. Aims: 1) To study the GASP phenotype in mixed cultures of *Escherichia coli* and *Salmonella enterica* serovar *typhimurium* wild type and strains harboring either the resistance to nalidixic acid (Nal^R) or to streptomycin (Str^R). 2). To evaluate the influence of multidrug resistance on the appearance of GASP phenotype. 3) To detect if there are genomic differences between both aged wild-type and used resistant mutants (Str^R and Nal^R) of *E. coli*, the pulsed-field gel electrophoresis (PFGE) of total genomic DNA was conducted.
Methods: In a typical GASP competition experiment, cells from a 10-day-old culture are inoculated as a numerical minority (1:100 vol/vol) into a young (1-day-old) culture. The genomic DNA from randomly chosen colonies was digested with *Sfi*I and resolved by pulsed-field gel electrophoresis (PFGE).
Results: In the mixture consisting of the aged *S. enterica* Str^R and young *E. coli* Nal^R, strong phenotype GASP mutants of *S. enterica* dominated after the third day of mutual growth. Likewise, but with inversed bacterial resistance, in the mixture of 10-day-old *S. enterica* Nal^R culture with young culture of *E. coli* Str^R, the *S. enterica* Nal^R GASP mutants of strong phenotype dominated the mixture after the fifth day and were maintained at about $1x10^8$ CFU/ml. Electrophoretic karyotype of the 10-days-old GASP mutants of *E. coli* strains carrying the resistance revealed additional bands when compared to the wild-type.
Conclusions: 1) The strong GASP phenotype was obtained in mixed cultures with the aged mutant strains, but not when the isogenic antibiotic-sensitive strains were used. 2) The cells in mixed cultures of double mutant *E. coli* strain Nal^R Val^R bear multiple useful GASP mutations that increase its fitness in comparison with mutants *E. coli* strain Str^R and *S. enterica* strain Str^R and showed strong GASP phenotype. 3) The PFGE analysis demonstrated the significant chromosomal rearrangements in 10-day-old bacterial antibiotic-resistant mutated cells that correspond with the appearance of strong GASP phenomenon.

AntiNeoplaston: Synthesis, Biological, and Clinical Evaluation in Egypt

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Background: Antineoplastons, first described by Burzynski, are naturally occurring peptides and amino acid derivatives, which control neoplastic growth. Antineoplaston A-10 (3-phenylacetyl amino-2,6-piperidinedione) is the first chem. identified antineoplaston. We previously reported the utility of antineoplaston-A 10 (3-phenylacetylamino-2,6-piperidinedione) as an endogenous cancer protector and immune modulator in breast cancer patients.
Methods: Antineoplaston A-10 level was measured in the urine of 31 breast cancer patients and 17 normal women using high performance thin layer chromatography (HPTLC).
Four new piperidinedione A 10 analogs were synthesized and tested for their antimitotic activity on a human breast cancer cell line against the prototype A 10 and the antibreast cancer drug tamoxifen. Moreover, the DNA binding capacity of such compounds. was evaluated against A 10.
Apoptosis was measured in patients with breast cancer at time of diagnosis and to correlate urinary antineoplaston A-10 levels with neutrophil apoptosis and to describe the direct effect of A-10 *in vitro* on neutrophil apoptosis in breast cancer patients. The participants were patients with a histologically confirmed diagnosis of breast cancer. Only those cases without previous treatment for breast cancer were included. Neutrophil apoptosis was assessed in breast cancer patients both morphology and by DNA fragmentation and studied relative to healthy controls. Antineoplaston A-10 was measured using high performance liqiuid chromatography in urine samples collected from the patients. Urine samples from normal women served as controls. Direct effect of antineoplaston A-10 on neutrophil apoptosis was tested *in vitro* after adding A-10 at a concentration. of 10 ng/mL to the cellular suspensions of breast cancer patients. Non-treated samples served as controls.
Results: Significantly lower antineoplaston A-10 levels were detected among patients with breast cancer. (E)-3-(4-Nitrocinnamoylamino)-2,6-piperidinedione and (E)-3-(4-hydroxycinnamoylamino)-2,6-piperidinedione were several-fold more potent antiproliferative agents than A 10 and tamoxifen. They also had significantly higher capacity to bind DNA than A 10. Conversely, (E)-3-(cinnamoylamino)-2,6-piperidinedione and (E)-3-(4-methoxycinnamoylamino)-2,6-piperidinedione had weaker biological profiles than the lead compound A 10. Detailed synthetic, spectroscopic, and biological data are reported.
Significantly higher neutrophil apoptosis levels were detected among patients with breast cancer with a P value <0.001. Urinary antineoplaston A-10 level is significantly neg. correlated with high apoptosis levels (P<0.0001). In vitro, antineoplaston A-10 was found to inhibit significantly the neutrophil apoptosis with a P value <0.0001.
Conclusions: Antineoplaston A-10 may provide rational basis for designing trials to employ its immune modulatory potentials as adjuvant therapy in breast cancer patients. These findings confirm the presence of immune defects among patients with breast cancer and such results should stimulate the development of new strategies to induce and augment immunity for the treatment of breast cancer.

Antibacterial, Antisecretory and Antihemorrhagic Activity of *Azadirachta indica* Used to Treat Cholera and Diarrhea in India

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Background: Indigenous uses of *Azadirachta indica* A. juss (Maliaceae) (locally known as neem) leaves in different parts of India for curing gastrointestinal disorder such as diarrhea and cholera is wide spread. The objective of the present study was to evaluate the antibacterial and antisecretory activity of neem extract against *Vibrio cholerae,* a causative agent of watery diarrhea such as cholera.
Methods: Methanol extract of neem leaves were tested using the strains of multi-drug resistant *V. cholerae* belonged to O1, O139 and non-O1, non-O139 serotypes. Antibacterial activity of the extract [10, 25, 50, 100, and 200 mg/ml (200 µl/well)] was determined by agar-diffusion assay. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were assessed using the broth microdilution method. The effect of the extract on fluid secretion and hemorrhage in intestine induced by *V. cholerae* was studied using mouse model (Male BalB/C mice, 23.8 ± 1.1 g body weight). Mice of each of groups 1 and 2 (n=2 per group in duplicated) were administered 500 µl of bacterial inoculums ($2×10^9$ CFU/ml) orally. After 1 h mice were fed either with 200 µl of methanol (group 1) or crude extract (group 2; at oral doses between 100 and 1800 mg/Kg in 200 µl of methanol) orally. Mice were sacrificed after 24 h incubation and fluid accumulation (FA) ratio was measured.
Results: Crude extract showed inhibitory activity against multi serogroup strains of *V. cholerae* by agar-diffusion assay with significance ($p<0.05$). MICs reached by 50% (MIC_{50}) and 90% (MIC_{90}), and MBC for the extract were 2.5 mg/ml, >5mg/ml, and 10 mg/ml respectively. Administration of the extract (1800 mg/kg) did not produce any sign of toxicity in mice. Group 1 mice showed fluid accumulation (FA ratio; between 0.11±0.01 and 0.16±0.02) and hemorrhage in the intestines. Neem extract showed activity with inhibition (of fluid secretion) values of 27.7 ± 7.8, 41.1 ± 3.4, 43.3 ± 1.3, 57.0 ± 5.9, and 77.9 ± 7.2 % at doses of 100, 200, 300, 450, and 1800 mg/kg respectively. Oral administration of the extract inhibited hemorrhage induced by *V. cholerae* in mouse intestine at a dose ≥300mg/kg (visually observed).
Conclusions: 1) Methanolic extract of *A. indica* leaves was an effective antibacterial agent against *V. cholerae,* and significantly reduced the fluid secretion and hemorrhage induced by *V. cholerae* in mouse intestine. 2) The active extract may be employed for the treatment of cholera and as potential source to develop novel antimicrobial compound and antisecretory drug useful to treat cholera and diarrheal patients.
Authors' disclosure statement: This work has been published in J. Ethnopharmacology: Thakurta, P., P. Bhowmik, S. Mukherjee, T. K. Hajra, A. Patra and P. K. Bag. 2007. Antibacterial, antisecretory and antihemorrhagic activity of Azadirachta indica used to treat cholera and diarrhea in India. J. Ethnopharmacology (Elsevier) 111 (3): 607-612.

The Frequency-Dependent Effect of Infrasound on Bull Sperm Velocity

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Background: The rheotaxic properties of sperm indicate the existence of specialized mechano-sensors in sperm membrane detecting the flow direction of the ejaculate. Therefore it is suggested that these sensors could serve as one of targets for infrasound (IS) effect and would have sensitivity to IS frequency. The overall aim of the present work was to study the frequency-dependent effect of 30dB IS on sperm velocity, ^{45}Ca uptake and intracellular level of c-AMP and c-GMP.
Methods: Experiments were performed on preliminary frozen bull sperm incubated in 2.9% Na-citrate water solution at 30°C. IS treatment of sperm samples was done by means of IS waves generating by a special setup. ^{45}Ca-uptake and intracellular cyclic nucleotides contents (cAMP and cGMP) were measured by Wallac 1450 liquid scintillation counter. The sperm velocity was recorded by means of digital video camera (SONY, Japan) set on biological microscope MB-14B connected to PC and sperm direct velocity was estimated by Pinnacle studio program.
Results: It was shown that 2Hz, 30dB 5min IS treatment caused the pronounced elevation effect (25%, ***: p<0.001) on the sperm velocity (Figure 1), which was accompanied by decreasing of intracellular level of c-GMP by 26±0.7% (*: p<0.05), and increasing of intracellular level of c-AMP 43.5±7% (*: p<0.05) and ^{45}Ca-uptake 285±6% (*: p<0.05) by sperm.

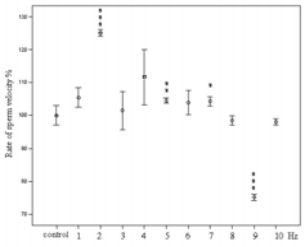

Fig 1. The effect of 1-10 Hz 30dB IS on bull sperm velocity. *: p<0.05; **: p<0.01; ***: p<0.001
Conclusions: The 2Hz, 30dB IS 5min pretreatment caused: 1) activation of sperm velocity; 2) increase of ^{45}Ca uptake, 3) increase of intracellular contents of cAMP and decrease of cGMP.

Synergistic Action of Resveratrol and its Polyhydroxylated Derivative with Cytarabine in HL-60 Human Promyelocytic Leukemia Cells

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Background: Resveratrol, a naturally occurring stilbene derivative, is a potent free radical scavenger exerting a multitude of biochemical and antineoplastic effects. Resveratrol was identified as an inhibitor of ribonucleotide reductase (RR), a key enzyme of DNA synthesis and an excellent target of cancer chemotherapy. Inhibitors of RR have been previously shown to exert synergistic combination effects with cytarabine, a well-established antileukemic agent. We investigated the biochemical effects of resveratrol and its polyhydroxylated derivative, 3,3',4,4',5,5'-hexahydroxystilbene (M8) on the *in situ* RR activity, cell cycle distribution, induction of apoptosis and inhibition of NF-kappaB. Furthermore, it was tested whether a combination of resveratrol or M8 with cytarabine could yield synergistic cytotoxic and apoptotic effects in human HL-60 promyelocytic leukemia cells.
Methods: Cytotoxic effects of resveratrol, M8 and cytarabine alone and in combination were analyzed using growth inhibition and clonogenic assays. Synergistic combination effects were identified by the Calcusyn software. *In situ* inhibition of RR was determined by the incorporation of ^{14}C-labelled cytidine into the DNA of resveratrol-treated HL-60 cells. Concentration of NTPs and dNTPs was measured by a HPLC method. Induction of apoptosis was studied using a Hoechst/propidium iodide staining method. Inhibition of TNF-alpha induced activation of NF-kappaB was shown by EIA and Western blotting and cell cycle distribution was analyzed by FACS.
Results: Resveratrol effectively inhibited incorporation of ^{14}C-labelled cytidine into DNA. Furthermore, incubation of HL-60 cells with resveratrol significantly decreased intracellular dCTP, dTTP, dATP and dGTP concentrations. M8 depleted intracellular NTP pools and dTTP as well as dATP pools. Moreover, M8 inhibited the activation of NF-kappaB and arrested HL-60 cells in the S-phase of the cell cycle. Based on these results, we investigated the combination effects of resveratrol and M8 with cytarabine. In growth inhibition, apoptosis and clonogenic assays, resveratrol and M8 acted synergistically with cytarabine in HL-60 cells.
Conclusions: Based on the synergistic cytotoxic and pro-apoptotic effects, the combination of cytarabine with resveratrol or M8 could become a viable option in the chemotherapy of leukemia and therefore deserves further testing.

The action of a new drug that targets a cancer's blood supply: the story of DMXAA (ASA404)

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DMXAA (ASA404; 5,6-dimethylxanthenone-4-acetic acid), developed in this laboratory [1,2], is currently undergoing Phase III clinical trial, in combination with the cytotoxic drugs carboplatin and paclitaxel, in patients with non small cell lung cancer. DMXAA was originally developed as the most active member of a series of analogues of the drug flavone acetic acid [3]. DMXAA has both a direct, early effect on tumour blood vessels, leading to inhibition of tumour blood flow, and a later effect that is mediated by local release of cytokines and other vasoactive molecules. It is this double action that maintains a sufficiently long duration of tumour blood flow arrest to induce irreversible damage and vascular collapse [1]. The effect DMXAA on tumour vascular endothelial cells appears to be mediated by p38 MAP kinase but the signalling pathway in tumour macrophages that leads to increased production of cytokines has not yet been identified. DMXAA facilitates a positive feedback loop where cytokines from tumour macrophages reduce blood flow and the resulting tissue hypoxia activates macrophages. The clinical antitumour activity of DMXAA is improved by co-administration of cytotoxic drugs and recent experimental results suggest that cytotoxic drugs can activate tumour macrophages through toll-like receptors such as TLR4. Thus, the potential antitumour activity of tumour macrophages may be increase firstly by DMXAA itself, secondly by hypoxia and thirdly by cytotoxic drugs. Studies with DMXAA may not only provide a basis for improved clinical therapy but also facilitate understanding of host events involved in the tumour response.

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Are Grapefruit, Orange, Lime, Pummelo and Apple the Forbidden Fruits of Drug Interactions?

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Background: Intestinal drug metabolism and carrier-mediated drug transport are accredited as fundamental factors affecting systemic drug availability. This presentation will initially review inactivation of CYP3A4 by grapefruit, Seville orange, lime and pummelo juices and increased drug bioavailability. The focus will be recent findings of inhibition of OATP1A2 mediated drug uptake by grapefruit, orange and apple juices and decreased absorption.
Methods: A series of in vitro and clinical pharmacokinetic studies were conducted.
Results: OATP1A2 was expressed in healthy human small intestine and co-localized with efflux transporter, MDR1 (P-glycoprotein), to the apical membrane of enterocytes. OATP1A2 was the only shown uptake transporter of the antihistamine, fexofenadine, which is also a substrate for MDR1. Grapefruit and orange juices at 0.5% normal strength halved the in vitro activity of OATP1A2. These juices at 10-fold higher concentration produced much less in vitro inhibition of MDR1, demonstrating potent and preferential in vitro inhibition of OATP1A2. Clinically, grapefruit and orange juice at high volume (1200 ml) markedly decreased oral fexofenadine bioavailability to 30% of that compared to water. Grapefruit juice consumed at a normal volume (300 ml) halved systemic fexofenadine availability consistent with a mechanism involving selective direct inhibition of intestinal OATP1A2. The major flavonoids in grapefruit and orange juice, respectively naringin and hesperidin, caused concentration-dependent in vitro inhibition of OATP1A2. The IC_{50} for naringin of 3.6 μM was several hundred-fold lower than that producing equal inhibition of MDR1. An aqueous solution of naringin (300 ml) at the same concentration as that measured in the tested grapefruit juice (1200 μM) decreased fexofenadine bioavailability to 75% of that with water, which accounted for half the reduction observed with grapefruit juice.
Conclusions: These results support a new mechanism of food-drug interactions. The fact that naringin was clinically active substance likely represented the first report of a single dietary ingredient producing a drug interaction in humans by modulating drug transport activity. This type of interaction appears relevant to a range of medications, particularly for those drugs that are essential for treatment of serious medical conditions.

Cytokine-associated neutrophil extracellular traps and antinuclear antibodies in Plasmodium falciparum infected children under the age of six

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Background: In *Plasmodium falciparum*-infected children, relationships between blood cell histopathology, blood plasma components, development of immunocompetence and disease severity remain poorly understood. Blood from Nigerian children with uncomplicated malaria was analyzed to gain insight into these relationships. This investigation presents evidence for circulating neutrophil extracellular traps (NETs) and antinuclear IgG antibodies (ANA). The presence of NETs and ANA to double-stranded DNA along with the cytokine profiles found suggests autoimmune mechanisms that could produce pathogenesis in children, but immunoprotection in adults.
Methods: Peripheral blood smear slides and blood samples obtained from 21 Nigerian children under six years of age, presenting with uncomplicated malaria before and seven days after initiation of sulfadoxine-pyrimethamine (SP) treatment were analyzed. The slides were stained with Giemsa and with DAPI. Levels of the pro-inflammatory cytokines IFN-γ, IL-2, TNF-α, CRP, and IL-6, select anti-inflammatory cytokines TGF-β and IL-10, and ANA were determined by immunoassay.
Results: The children exhibited circulating NETs with adherent parasites and erythrocytes, elevated ANA levels, a Th2 dominated cytokine profile, and left-shifted leukocyte differential counts. Nonspecific ANA levels were significant in 86% of the children pre-treatment and in 100% of the children seven days after SP treatment, but in only 33% of age-matched control samples collected during the season of low parasite transmission. Levels of ANA specific for dsDNA were significant in 81% of the children both pre-treatment and post-treatment.
Conclusions: The results of this investigation suggest that NET formation and ANA to dsDNA may induce pathology in falciparum-infected children, but activate a protective mechanism against falciparum malaria in adults. The significance of in vivo circulating chromatin in NETs and dsDNA ANA as a causative factor in the hyporesponsiveness of CpG oligonucleotide-based malaria vaccines is discussed.

A Novel Anti-Angiogenic Glycotherapeutic for Breast Cancer

BANERJEE DK

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Complex etiology of breast cancer needs personalized treatment. Angiogenesis is critical for the breast tumor growth. Our objective has been to evaluate the requirement of lipid-linked oligosaccharide (LLO), a pre-requisite for asparagine-linked glycoproteins in angiogenesis and ask if anti-angiogenic glycotherapeutics could eliminate the breast tumor growth. Our angiogenesis model is a non-transformed capillary endothelial cell line with preserved physiology and anatomy responding adequately to cellular microenvironment. cAMP-signaling enhances LLO biosynthesis and results in accelerated cell cycle progression, increased capillary lumen formation and HSP-70 and HSP-90 expression. Mannosylphospho dolichol synthase (DPMS), a key regulator in the LLO biosynthesis is also increased. Cloning and sequencing identifies a PKA motif in DPMS gene and supports PKA-dependent phosphorylation. On the other hand, tunicamycin (TM, an antibiotic and an LLO inhibitor) inhibits the cell surface N-glycan expression, down regulates the expression of Bcl-2, D-type cyclins, cdks and the transcription factor E2F causing a G1 arrest and halts angiogenesis. Increased caspase-3, -9 and -12 expression, DNA laddering and Annexin V binding all support apoptosis. High expression of GRP-78/Bip indicat "ER stress" and the induction of unfolded protein response. DPMS activity as well as its protein expression is lost, and $VEGF_{165}$ looses the cellular protection in TM treated cells. TM inhibits angiogenesis in Matrigel™ implants and also reduces the humanized breast tumor growth in athymic nude mice by 50-65% in 1-3 weeks when administered orally or intravenously. Therefore, TM has a potential to succeed in the clinic as an excellent breast cancer therapeutic.
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Search for diagnostic and prognostic markers for Glioblastoma multiforme(GBM).

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Background: Glioblastoma Multiforme are brain tumors which are a heterogeneous group of central nervous system neoplasms that arise within or adjacent to the brain. Some are curable by surgical resection, but many cannot be eradicated by current treatments, and, when they are, disabling neurological injury often ensues. At present these brain tumors are detected by imaging only after the onset of neurological symptoms and no early detection strategies are in used. Imaging, the obvious screening strategy for glioblastomas is extraordinarily costly, especially given the relative rarity of these tumors in comparison with breast or prostate cancer. Genetic testing is a better option and is desirable as a screening tool because it is generally based on a simple blood test. The current histopathological approach to the diagnosis and classification of glioblastomas is not satisfactory in many respects.Therefore,a gene and protein based diagnostic and classification system is best for prognosis and therapeutic approaches.
Methods: The following techniques were used to analyse glioblastoma specimens and cell lines and search for biomarkers a)Microarray analysis b)SELDI studies c) Laser Optical tweezers d)DIGGE and e)Real Time PCR f)ELISA.
Results: Several novel proteins and genes were identified that differentially express in Glioblastoma specimens in comparison to normal control brain samples.
Conclusions: There are several genes that are either down regulated,loss of function or upregulated that we observed in our research.We are working farther on the importance of these genes and their proteins in their role as a significant biomarker by analyzing more patient population.

Synthesis and Biological Evaluation of Anticancer β-Lactams

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Background: Systematic analyses of polyaromatic derivatives through an examination of their anticancer effects *in vitro* and *in vivo* has been demonstrated. During the course of our studies, it has been anticipated that conformationally restricted analogues might increase the potency. We have envisioned that stereodefined β-lactams will serve as improved conformationally constrained analogues of our open-chain diamides that have already been shown to possess anticancer activity. Since many β-lactams (penicillin-types of compounds) have been widely accepted as safe antibacterial agents for more than 50 years, our hypothesis is that novel drugs can be synthesized and selected within this class that will have both an enhanced anticancer activity and low toxicity to normal tissues.
Aims: 1) To synthesize β-lactams derived from polyaromatic imines. 2) To study their effects *in vitro* and *in vivo*. 3) To study the mechanism of action of the lead β-lactams
Methods: Synthesis of β-lactams was achieved following cycloaddition reaction of imines with acid chloride in the presence of triethylamine. *In vitro* cytotoxicity and *in vivo* study were performed using NCI protocol. Ames assay, topoisomerase inhibition and cell cycles were also investigated.
Results: A number of racemic and optically active novel β-lactams were synthesized. Synthesis of these agents using microwave irradiation was performed. An unprecedented stereochemical results was identified. Some of these compounds demonstrated promising antitumor activity *in vitro* and in animal tumor model systems. In some instances the anticancer activity exceeded that of cisplatin *in vitro*. These agents demonstrated a blockade of the G_2/M checkpoint in cancer cell lines, but they had no effects on topoisomerases. The active compounds were non-mutagenic.
Conclusions: 1) Synthesis of several novel anticancer β-lactams achieved. 2) One of them demonstrated antitumor activity in animal model. 3) Although the mechanism of action of the lead compounds was not established, our research on cell cycle analysis offered intriguing possibilities.

Synthesis of a Series of 8-(Substituted-Phenyl)xanthines and a Study on the Effects of Substitution Pattern of Phenyl Substituents on Affinity for Adenosine A_1 and A_{2A} Receptors- The Magic of Theophylline

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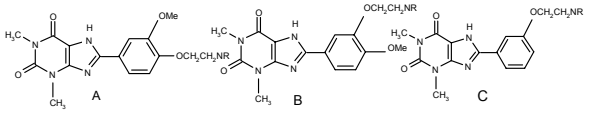
Background: Adenosine receptors are promising therapeutic targets in a wide range of conditions, including cerebral and cardiac ischaemic diseases, sleep disorders, immune and inflammatory disorders and cancer. 8-Phenyltheophylline is the parent member of a variety of potent adenosine receptor antagonists. Herein we report the synthesis of a new series of 8-(substituted-phenyl)xanthines and the effects of substitution pattern of phenyl substituents on adenosine receptors binding affinity.
Methods: The three series of compounds A, B and C were prepared by treating 5,6-diamino-1,3-dimethyluracil with alkylaminoalkyl substituted derivatives of vanillin, isovanillin and 3-hydroxybenzaldehyde and subsequent cyclization in thionyl chloride.



Results: The compounds were evaluated for their affinity for A_1 and A_{2A} receptors using [^{3}H]DPCPX and [^{3}H]ZM-241385 as radioligands. Table summarizes the observed affinities of various newly synthesized 8-phenylxanthine derivatives in radioligand binding assays at human A_1 and A_{2A} receptors. The presence of a methoxy substituent at 3 or 4- position of phenyl ring in A and B along with an *ortho* polar side chain increases selectivity for A_2 over A_1 receptors. However absence of a methoxy group as in C results in almost equal selectivity for both subtypes.

Table: Adenosine A_1 and A_{2A} binding affinities of compounds

Comp. No.	A	B	C	DPCPX	ZM 241385
A_1 K_i (μM)	>100	>100	2.1	0.095	0.54
A_{2A} K_i (μM)	0.7	2.7	0.8	0.13[1]	0.064

Conclusions: It can be concluded that suitable selection and positioning of aryl substituents may lead to development of potent and selective xanthine based adenosine receptor antagonists.

Bacteriocin substance producting by *Lactococcus lactis* against *Listeria monocytogenes* isolated in ready-to-use fish fillets

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Background: Bacteriocins produced by lactic acid bacteria are important in preventing the growth of pathogenic bacteria especially in ready to use fish that could be potentially contaminated by pathogens.
Methods: 26 *Carnobacterium piscicola*, 5 *Carnobacterium divergens* and 24 *Lactobacillus* spp. strains were screened against 22 strains of *Listeria monocytogenes*, 5 strains of *Yersinia enterocolitica*, 1 strain of *Staphylococcus aureus*, 1 strain of *Pseudomonas* spp, 1 strain of *Salmonella* spp. All producer and indicator strains were isolated from farmed gilthead fish fillets (*Sparus auratus)* held in the culture collection of Department of Zootechical Sciences. The microorganisms were previously identified by biochemical method and submitted to antagonistic activity experiments in solid and liquid media by using the spot on lawn and the agar well diffusion assay, respectively. LAB strains producers of bacteriocin-like substance in solid media, were then tested for bacteriocin producing in broth and sensitivity to heat (60-80°C/1h; 100°C/30 min.; e 121°C/10 min), and to proteolitic enzymes. Bacterial growth curves and bacteriocin production were studied for LAB strain producer.
Results: On 25,4% of LAB strains showing antimicrobial activity in solid media, only 1 strain, belonging to *Lactococcus lactis* Sa 31, was able to produce bacteriocin in liquid medium. *Lact. lactis* Sa31 showed a narrow spectrum of it activity towards *Listeria monocytogenes* (clear inhibition zones 5-8 mm). No inhibition zones were observed over *Staphylococcus aureus*, *Salmonella* spp., *Pseudomonas* e *Yersinia enterocolitica*. The increased bacteriocin production of *Lact. lactis* Sa 31 was observed after 11 h of incubation at 30°C in Elliker agar with maximum titre of bacteriocin of 2,560 Arbitrary Units ml^{-1}. The bacteriocin was sensitive to trypsin, pronase E and papain and was inactivated by temperatures ≥ 100°C.
Conclusions: The bacteriocin produced by *Lactococcus lactis* showed a relevant antimicrobial activity against different strains of *Listeria monocytogenes* and could be employed as biopreservative in ready-to-use fish fillets.

The Modified Vaccination Technique developed by Barabas provides the "magic bullet" for the prevention and cure of chronic ailments such as autoimmune disorders and cancer specifically, without side effects

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Background: Barabas has developed and implemented a new vaccination method called modified vaccination technique (MVT) - in experimental animals - that can be employed both prophylactically and therapeutically with equal effectiveness to combat chronic disorders such as autoimmune diseases, cancer and exogenous antigen induced mishaps. This breakthrough discovery of antigen presentation by the MVT allows the induction of a predetermined beneficial immune response outcome.
Presently available vaccination programs are preventative (through active immunization) or therapeutic (through passive immunization) and neither technique can evoke specific downregulatory (in autoimmune disorders) or upregulatory (in cancer and chronic infections) immune responses to correct chronic disorders.
Methods: When vaccinating, using the MVT, appropriately assembled immune complexes - made up of the specific target antigen and specific homologous polyclonal or monoclonal antibodies against the target antigen - have to be prepared (the "magic bullets") for the prevention and treatment of presently drug only treatable conditions such as autoimmune diseases and cancer. The goal is to inject exactly the right immune complex (the "magic bullet") into recipients to evoke the production of the same class of immunoglobulin (i.e., antibody) with the same specificity against the target antigen that resides in the inoculum.
Results: Using the MVT antibody information transfer is achieved resulting in predetermined immune responses. Through the utilization of the MVT the potential of downregulating or upregulating immune events - in humans with certain autoimmune disorders and cancer - to achieve self cure and regained tolerance to self is in sight.
Conclusion: We believe absolutely in the immune system's natural ability to correct mistakes. It requires only in certain instances tailor made "instructions" for eliciting and maintaining redirected beneficial immune-response outcomes.

Allosteric modulation of hormone receptors in cancer treatment

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Background: Trilostane was originally developed and used as an inhibitor of 3-beta hydroxysteroid dehydrogenase in treating Cushing's syndrome. It was later found to confer clinical benefit in breast cancer patients. Our studies began with the observation that trilostane (at micromolar concentrations) could effect the function of the estrogen receptor (ER) but without displacing the native ligand, estradiol, from its ligand binding site. We have proposed that trilostane can act as an allosteric modulator of the ER and our research has focused on trying to find an explanation for its efficacy in the treatment of postmenopausal breast cancer.
Methods: In these studies we have used a wide range of biochemical and functional methods, including ligand-binding assays, gene reporter assays, gene expression microarrays and cell proliferation assays, in order to try to define an alternative mechanism of action for trilostane in the context of breast cancer.
Results: Trilostane does not displace radiolabelled estradiol from estrogen receptor preparations. It inhibits MCF-7 breast cancer cell proliferation at concentrations of 1μM and above. It has direct inhibitory effects on ER function and events mediated through oestrogen response elements (EREs) and activating protein-1 motifs. In a study of the effects of trilostane and tamoxifen, on MCF-7 cells using microarrays, striking differences were found in gene expression profiles. Interestingly, trilostane was found to selectively up-regulate the expression of the beta subtype of estrogen receptor (ERβ), an effect replicated *in vivo*. This is significant as tamoxifen-resistant breast cancer is often associated with reduced ERβ protein expression and trilostane can inhibit proliferation of tamoxifen-resistant MCF-7 cells *in vitro*.
Conclusions: Our data support previous clinical findings that trilostane can influence the effectiveness of tamoxifen and may subvert tamoxifen resistance, and demonstrates in a wider context that allosteric modulation of receptor function may be a useful therapeutic approach in circumstances in which the receptor ligand binding domain is occupied by its cognate ligand or an antagonist. This concept has potential application in clinical situations in which either nuclear receptors or surface membrane located receptors are implicated in cancer progression.

Elesclomol: A Novel Oxidative Stress Inducer for the Treatment of Metastatic Melanoma

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Background: Elesclomol (E) is a small molecule drug candidate that stimulates production of reactive oxygen species (ROS) and selectively induces cancer cell apoptosis. The clinical efficacy of E in combination with paclitaxel (P) was investigated in a double-blind, randomized, controlled Phase 2 trial in melanoma.
Methods: The *in vitro* activity of E was assessed in human Hs294T melanoma, Ramos lymphoma and HSB2 leukemia cells. Preclinical efficacy was tested in an M14 melanoma human xenograft model in mice. Stage IV metastatic melanoma patients were randomly assigned to 213 mg/m^2 E plus 80 mg/m^2 P (E+P) or 80 mg/m^2 P as a weekly one-hour intravenous infusion, three of every four weeks until disease progression per RECIST or death. The primary efficacy endpoint was progression-free survival (PFS).
Results: E rapidly increased ROS production in cancer cells. Sustained oxidative stress led to activation of the intrinsic mitochondrial apoptosis pathway, with cardiolipin oxidation, decreased mitochondrial membrane potential, cytochrome c release and caspase activation. Antioxidants blocked all activities of E. ROS increase and apoptosis preferentially occurred in cancer cells relative to normal cells. E inhibited tumor growth as a single-agent and synergized with P in causing tumor regression in human xenograft tumor models. At 21 US sites, 53 subjects were randomized to E+P and 28 subjects to P. The addition of E to P yielded a doubling of median PFS (112 *v* 56 days) and a 41.7% risk reduction for disease progression/death (hazard ratio = 0.583, $P = 0.035$). Respective response rates were 15% *v* 3%. Median overall survival was 11.9 *v* 7.8 months. E+P was well tolerated.
Conclusions: 1. E increased ROS levels in cancer cells, leading to apoptosis. Cancer cells produce elevated ROS relative to normal cells, and are therefore susceptible to further ROS increases which push the cell beyond its oxidative stress tolerability limit. In this way, E takes advantage of a fundamental hallmark of cancer to selectively kill cancer cells with little effect on normal cells. 2. In a randomized, blinded Phase 2 clinical trial, E+P resulted in a clinically meaningful, statistically significant increase in PFS, with an acceptable toxicity profile and encouraging survival data. A global Phase 3 trial (SYMMETRY^{SM}) of E+P *v* P in chemotherapy-naïve stage IV metastatic melanoma patients is ongoing.

Blood-brain barrier P-glycoprotein function in aging and neurological disease: *in vivo* measurements in humans with [^{11}C]-verapamil PET.

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Introduction: The blood-brain barrier (BBB) possesses a highly concentrated transporter, the P-glycoprotein (P-gp) transporter. It protects the brain from several endogenous and exogenous compounds by acting as an active cell membrane efflux pump. Changes in P-gp function have been implied in several neurological conditions. Upregulation of P-gp has been found to prevent uptake of drugs in brain tumours and in epilepsy, leading to therapy-resistance. Decreased P-gp function has been associated with Amyloid-β transport and progression of Alzheimer's disease (AD). Also, it has been related to toxin exposure and the development of Parkinson's disease (PD). Finally, age-associated decline in P-gp function may facilitate the accumulation of toxic substances in the brain. We studied *in vivo* BBB P-gp function in older healthy subjects and in patients with PD.
Methods: BBB P-gp function was studied using positron emission tomography (PET) and [^{11}C]-verapamil. 17 healthy volunteers with age 18-86, 22 patients with Parkinson's disease in different disease stages and six subjects with parkinsonism (PSP and MSA) were scanned. Distribution volume (DV) of the tracer in the brain was calculated using Logan analysis. Statistical Parametric Mapping (SPM2) was used to study specific regional differences between the subject groups.
Results: Older subjects showed significantly increased brain tracer uptake as compared to the younger healthy subjects, indicating decreased BBB P-gp function. In PD, the more advanced patients showed increased tracer uptake in frontal regions, while de novo patients showed decreased uptake. In patients with parkinsonism, increased uptake in basal ganglia regions was observed.
Conclusion: Decreased blood-brain barrier P-gp function with aging could be a mechanism by which age acts as the main risk factor for the development of neurodegenerative disease. It also means that several drugs will reach higher concentrations in the brains of elderly patients. The knowledge that P-gp function may be modulated by drugs is also important, as P-gp modulators may provide opportunities for intervention, not only in treatment resistant cancer or epilepsy but also in the progression of neurodegenerative diseases.

Laser-based Stable Isotope Tracing in Human Breath

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Background: Stable isotopes have become widely available at affordable prices. They are increasingly used as tracers in clinical research and diagnosis. In breath analysis, analytical methodology has relied upon expensive and cumbersome instrumentation such as isotope ratio mass spectrometry (IRMS). In the last decade, isotope ratio infrared spectrometry has become a serious competitor to IRMS. Lasers have recently been employed to measure the $^{13}C/^{12}C$ isotope ratio of exhaled carbon dioxide. However, breath samples must be prepared to reduce the water vapor content. In this work, we measured—without sample preparation—the D/H isotope ratio increase in exhaled water vapor following the intake of a deuterated tracer (D_2O).
Current sensitive trace-gas detection schemes for infrared laser spectroscopy cannot handle condensable vapors. For this purpose, we developed a high-temperature multipass cell.
Methods: A healthy volunteer drank 5.14 mL of 99.9 % pure D_2O diluted in 200 mL tap water. The D_2O intake corresponds to a dosage of 16 mg deuterium/kg body weight, a safe level that is within the dosage range used in clinical studies. Breath samples were then collected at 24-hour intervals in a commercial bag heated above 323 K until original background levels were obtained. The high-temperature multipass cell was filled with a collected breath sample without any preliminary sample preparation. Roto-vibrational spectra were recorded by finely tuning a mid-infrared laser between 2788.60 and 2789.80 cm^{-1}.
Results: The D/H isotope ratio is usually expressed in parts per thousand relative to a standard, using the common notation $\delta^2H = (R_{sample}-R_{standard})/R_{standard} \times 1000$, where R is the ratio of the heavier to the lighter stable isotope of hydrogen. We performed repetitive measurements before D_2O intake and determined δ^2H with a standard deviation of 23 ‰. Following D_2O intake, we measured a significant δ^2H increase in exhaled breath of 768 ‰. We will present time-dependent measurements after D_2O intake and derive the total body water.
Conclusions: Following the ingestion of only 5 mL D_2O, an infrared laser spectrometer determines — without sample preparation — the D/H isotope ratio increase in exhaled water vapor for the first time. This opens the door to many clinical applications. The laser spectrometer has a great potential to become a portable and affordable device.

Erythromycin: Magic Bullet for Sore Throat and its Consequences in Children

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Background: Most people suffer from sore throat particularly in the first two decades of life. Previous studies have proved that sore throat is a major cause of sickness and a potentially dangerous source of infection in children. We evaluated the clinical situation of sore throat in Nepalese children, and reviewed literature to assess erythromycin as its magic remedy.
Methods: We prospectively evaluated the clinical data of children below 19 years who visited Children's Medical Diagnosis Center and Durga Bhawani Polyclinic from January 2006 to December 2007. All patients were consulted and the final diagnosis was made by a consultant pediatrician and pediatric cardiologist. We also analyzed all the publications indexed in Pub Med upto June 27, 2008 on key words *children and sore throat and erythromycin* conducted in various studies.
Results: Of the total 1175 examined patients 159 (13.53%) had tonsillitis. Amongst 159 patients 63.53% were between 4 to 10 years. The male female sex ratio was 1.48. Our clinical experience supplemented by reported 150 literatures have showed that erythromycin has been used in all age groups and sex, is effectively acceptable, well tolerated orally, and also available in parenteral form. It has been available worldwide after its discovery by McGuire and coworkers in 1952 from a strain of Streptomyces erythreus, originally obtained from soil in the Philippines. It is able to eradicate a broad range of bacteria including streptococci, staphylococci, listeria, legionella, diphtheria, pertussis, tetanus, syphilis and

Camphylobacter jejuni without any fatal toxic effect. It exerts effective concentration on tonsilar tissues. Erythromycin has both curative (e.g. sore throat) and prophylactic (e.g. rheumatic fever, rheumatic heart disease, acute glomerulonephritis) benefits in man. It has been proved effective in treating sore throat and other infections thereby decreasing its untoward life threatening cardiac, renal, respiratory and neuronal complications, as well as the cost of treating cardiac consequence, such as valvular surgeries.
Conclusions: Erythromycin has reliable chemotherapeutic effect in treating sore throat and preventing its cardiac, respiratory, renal and neuronal consequences in children of developing and industrialized nations thus proving its role as a 'magic bullet'.

Application of Saffron and Its Ingredients as a Known Pharmacological Herb from Ancient Times and the Mechanisms of Their Action

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Background: Saffron (*Crocus sativus* L.), which is named as "*red gold*" in Iran, has been used not only as a food additive and a dye, but also as a medicinal herb in most parts of the world from ancient times. In the last two decades, it has been reconsidered because of its various biological properties. Chemical analysis has shown the presence of more than 150 components in saffron stigmas. In this presentation we reviewed the historical and medicinal uses of saffron in different parts of the world, especially in Iran where saffron was cultivated for the first time (in addition to the wild type), and where nowadays is the biggest saffron producer (more than 80% of world saffron cultivation). Then, the new findings from our laboratory as well as other research groups, about its medicinal properties and various cellular and molecular mechanisms of action were discussed.
Methods: Web of Science and Medline were searched for saffron and its constituents. For ancient uses of saffron, manual searching of Persian books and searching internet resources, among with manual searching of their references were done. In our experiments, after purification of important constituents from Iranian saffron, their application on breast and gastric cancer were studied in model animals; their molecular mechanism of action was also investigated using *in vitro* experiments.
Results: Saffron was known from more than 3000 years ago by Iranians, Assyrians Babylonians and Minoans; not only for its application as a food additive, dye and perfume, but also for its usage as a medicinal herb (alone or in combination with other drugs) to treat a wide range of diseases. Newly, its application in a variety of disorders involving neuronal, cardiovascular and other systems as well as cancer has been investigated. The cellular and molecular mechanisms of its action are also under study. Saffron's more powerful components are carotenoids and monoterpene aldehydes. Structure-function relationship studies show that some properties are related to deglycosylated derivatives, while others belong to more glycosylated ones.
Conclusion(s): Saffron as an important medicinal herb is a good candidate to be considered for new drug design.

Ear Surgery – Place for Topical use of Mitomycin C

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Background: Stenosis and atresias of the external auditory canal (EAC) are rare conditions difficult to manage with success. The main reason to operate is severity of the hearing impairment and threatening cholesteatoma of the EAC. Some authors do not find classical surgical methods sufficient and they favor the use of KTP/532 laser, or larger surgical procedures. The proven ability of Mitomycin C (MMC) to inhibit fibroblasts *in vitro* has stimulated its use it in treatment to prevent stenosis and adhesion, also in otorhinolaryngology. The objective of the study was to evaluate the opening of the external auditory canal in fibrotic atresias (congenitally and secondary) and the hearing improvement, after the surgery and concomitant use of topical MMC.
Methods: Ten patients, all together fourteen ears, with fibrotic external auditory canal (EAC) atresias due to chronic external otitis, post-traumatic, post irradiated, or congenital cause were included. During the surgical procedure – meatoplasty only endaural approach was used, and 1 mL of MMC (0.4 mg/mL) was applied for 4 minutes to the EAC. In 5 ears the application of MMC was repeated one to six month later, than 1mg/mL concentration of MMC was used. During the application of MMC the tympanic membrane was protected with a thin layer of dry Gelfoam. Audiometric evaluation included pre and postoperative air-conduction thresholds (AC) and bone-conduction thresholds (BC).
Results: Between 9 and 56 months after the surgery and concomitant application of MMC the microscope visual control of opening of EAC was assessed. The hearing improvement was observed by using preoperative and postoperative pure tone threshold audiograms (PTA). In 10 ears (72 %) the ear canals reminded open with a postoperative air-bone gap of 10 dB or less. No sensorineural hearing loss was detected after surgery.
Conclusions: 1) MMC used on a limited number of ears during surgery was effective in preventing scaring in fibrosis ending with atresias of EAC. 2) No complications or sensorineural hearing loss were encountered after surgery and MMC application. 3) The results of our study are comparable with the published reports and according to the follow up period our outcome results with a success rate of 72% could be considered as final.

Cefquinome and Amoxicillin in Goats: PK/PD Integration

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Background: Cefquinome (CFQ) and amoxicillin (AMO) are beta-lactam antibiotics, belonging to the groups of cephalosporin and penicillin, respectively. CFQ and AMO are intended for the treatment of respiratory infection diseases, caused by *Mannheimia haemolytica* and *Pasteurella multocida* and for the treatment of mastitis in livestock. The aim of this study was to obtain and integrate pharmacokinetic (PK) and pharmacodynamics (PD) data of CFQ and AMO in the goats.
Methods: The pharmacokinetic profile of CFQ and AMO in goats was investigated following intravenous (i.v.) and intramuscular (i.m.) administration at the doses of 1 mg/kg and 15 mg/kg, respectively. At the same time, the minimum inhibition (MIC) and minimum bactericidal (MBC) concentrations of CFQ and AMO against two reference strains of *Mannheimia haemolytica* and *Pasteurela multocida* were determined in cation-adjusted Mueller-Hinton Broth (MHB) and goat blood plasma. The bactericidal activity of CFQ and AMO was tested against *Mannheimia haemolytica* and *Pasteurella multocida* using the time killing method.
Results: After i.v. administration of CFQ, the pharmacokinetics of CFQ indicated a small volume of distribution (Vss=0.204±0.02 L), a rapid clearance (2.433±0.59 mL/min) and half-life of 1.36 ± 0.2 h. After i.v. administration of AMO, the short terminal half-life ($t_{1/2z}$) of 2.0 ± 0.47 h was the net result of ratio of the relatively small volume of distribution to the total clearance. After i.m. dosing absorption of CFQ was complete (F≥100) and rapid and terminal half-life was 1.54±1.4 h. However, after i.m. administration of AMO, the half-life in goats (7.89±2.26 h) was much higher than after i.v. administration of AMO. The difference in half-lives between i.v. and i.m. administration of AMO suggest that the i.m administration of AMO in goats produce a flip-flop phenomenon.
In broth MIC and MBC of AMO against *M. haemolytica* were 0.188 μg mL^{-1} and against *P. multocida* were 0.250 μg mL^{-1}. MIC and MBC of AMO against *M. haemolytica* in goat plasma were MIC=MBC=0.188 μg mL^{-1} and against *P. multocida* in blood plasma of goats were MIC=MBC=0.375 μg mL^{-1}. MIC and MBC of CFQ, when determined in MHB, were 0.047 μg mL^{-1}, against *M. haemolytica* and *P. multocida*. MIC and MBC of CFQ against *M. haemolytica* in goat plasma were 0.094 μg mL^{-1}. The respective value against *P. multocida* was MIC= 0.047 and MBC= 0.094 μg mL^{-1} in goat plasma. MIC and MBC data against two reference strains *M. haemolytica* and *P. multocida* indicated that the AMO generally exhibited higher MIC and MBC when compared with CFQ. CFQ and AMO were shown to be time dependent bactericidal antibiotics against target pathogens and the killing occurring at a concentration close to the MIC. The rate of killing was not significantly influenced by the increase of antibiotic concentration.
Conclusions: Taking into consideration the PK and PD parameter of AMO in goats, the concentration of the drug in the plasma remain above the MIC (=0.375 μg/mL) of *M. haemolytica and P. multocida* in the goats for 12 hours after i.m. administration, and it can be concluded that a once-daily amoxicillin i.m. administration at the dose of 15 mg/kg b.w, yields therapeutically effective drug levels. Furthermore, according to PK analysis and PD data of CFQ, it can be concluded that, for susceptible bacteria, a twice-daily cefquinome i.m. administration, at the dose of 1 mg/kg b.w., will yields therapeutically effective drug levels.

Cell-free octameric hemoglobin as a blood substitute

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Background: Considerable progress has been made in the development of red blood cell substitutes, in particular with hemoglobin (Hb) based oxygen carriers designed to correct oxygen deficiency. The two major problems are encountered with acellular Hb in the plasma: the optimum oxygen affinity for adequate oxygen delivery to tissues and the dissociation of Hb tetramers ($\alpha_2\beta_2$) into dimers ($\alpha\beta$). Clinical trials with different Hbs obtained either by chemical modification or by protein engineering to prevent tetramer dissociation, have shown a vasoactivity in plasma. Increasing the molecular size of the carrier has been proposed to reduce the undesirable vasoactive properties. In order to eliminate problems related to the small size of acellular oxygen carriers, we have made the next logical step of going from a tetramer to an octameric form of Hb by introducing the cysteine residues oriented towards the exterior of the β-subunits.
Methods: The mutation βG83C was introduced by site-directed mutagenesis into the co-expression vecteur pHE7 containing human α, β-globin cDNAs and an E. coli methionine aminopeptidase cDNA. The recombinant Hb βG83C (rHb βG83C) have engineered in JM 109 strains of *E.coli* and purified by ion exchange chromatography.
Results: The analysis of purified rHb βG83C by exclusion size chromatography shows the presence of a single molecular species corresponding to a molecular weight of 129 kDa *ie* a dimer of tetramer. The CO rebinding kinetics after flash-photolysis of this octameric rHb are similar to that of tetrameric Hb with two phases corresponding to two allosteric states (R and T states). The different studies show that this octameric rHb was stable and did not dissociate easily into small molecular species at low concentration. This rHb was resistant toward potential disulfide reducing agents present in fresh human plasma. Moreover these octamers does not interact with haptoglobin, a plasma glycoprotein that binds dimers to participate in the elimination of Hb from blood circulation.
Conclusions: This molecule is thus potentially useful as blood substitutes. The objectives are now to evaluate the effects of this rHb on the biology of the endothelial cell.

Inhaled H_2S for suspended animation in anesthetized and ventilated mice

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Background: Several organisms can reversibly slow down their vital functions in order to sustain otherwise lethal environmental stress, a phenomenon called hibernation. In mice, inhaling hydrogen sulfide (H_2S) induced such a hibernation–like state called „suspended animation" with decreased heart rate, respiratory minute volume and metabolic expenditure, and consecutively hypothermia[1]. H_2S also is an endogenous gaseous messenger and signaling molecule, and both pro- and antiinflammatory effects were reported. Since in rodents anesthesia per se causes hypothermia, we tested the hypothesis whether inhaled H_2S may also induce suspended animation in anesthetized, mechanically ventilated and instrumented mice.
Methods: 15 hours after laparatomy animals received 100 ppm H_2S or vehicle for 5 hours with core body temperature maintained at 38° or 27°C. Left ventricular pressure-volume loops were assessed using a pressure-conductance catheter. Cytokine (TNF-α, IL-6) and chemokine (MCP-1, MIP-2) formation, myeloperoxidase and NF-κB activation were measured in lung homogenates, mitochondrial respiration was measured in liver biopsies (high-resolution respirometry).
Results: In contrast to awake mice, H_2S alone did not affect hemodynamics. Within 3 hours, however, both hypothermia alone and combined with inhaled H_2S decreased blood pressure, heart rate and thus cardiac output, while stroke volume, ejection fraction and end-diastolic pressure were unchanged. While inhaled H_2S and hypothermia alone comparably attenuated lung chemokine tissue levels, only combining hypothermia and H_2S significantly decreased tissue IL-6 levels. Strikingly, H_2S significantly increased NF-κB activation during normothermia. Combining hypothermia and inhaled H_2S attenuated the cytochrome-c-induced stimulation of mitochondrial respiration.
Conclusions: 1) In contrast to awake mice anesthesia blunted the hemodynamic effects of inhaled H_2S alone. 2) H_2S has anti-inflammatory properties beyond hypothermia alone. 3) Reduced cytochrome-c-induced stimulation of mitochondrial respiration suggests attenuated mitochondrial damage.

References: 1. Blackstone et al, Science 2005;308:518

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Light (LM) and electron microscope (EM) examination of the effects of methotrexate (MTX) on the endosalpinx.

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Background: To examine the effects of increasing doses of mtx on the fallopian tubes.
Methods: The study was carried out on 24 female rats (Albino Wistar type, 250-300 g). Different doses of Mtx were given to the randomly divided 4 groups by IP injection: 1mg/kg to group 1, 5mg/kg to group 2, 10 mg/kg to group 3 and physiological serum to group 4 as the control group. After ten days, the fallopian tubes of the rats were removed for examination separately by LM and EM for comparison.
Results: LM showed that in group I the surface epithelial cells were normal and the lamina propria was infiltrated by numerous inflammatory cells with a prevalence of polymorphonuclear leucocytes. Findings in groups 2 and 3 were similar: the lamina propria was infiltrated with granulocytes in one specimen from each of the two groups, and granulocytes were also observed among epithelial cells. In the control group all surface structures were found to be in a normal condition. EM showed cilial loss in the epithelial cells and central crystolysis in mitochondria in all group 1 specimens. Findings in groups 2 and 3 were similar. The cytoplasm of the epithelial cells seemed to be dense, there was prominent crystolysis in the mitochondria, and vacuolisation in the cytoplasm seemed to be augmented. Cilial loss was prominent, and the basal membrane was irregular. Epithelial cell nuclei were in disorder. Lipid granules were observed extensively in epithelial cells. Eosinophils seemed to be dominant in connective tissues below the epithelium. In all control group specimens the epithelium seemed to be normal with all organelles in place; the condition of intercellular junctions, ciliated epithelium and all mitochondria also seemed to be normal, and the basal membrane was observed to be in order.
Conclusion: The ultrastructural derangements resulting from administration of Mtx in doses in excess of 1mg/kg can cause a reduction in the surface epithelium's ability to make rhythmic lashing movements and can impair the patency of the fallopian tubes. All these disturbances could be involved to some degree in the causation of infertility and recurrent ectopic pregnancy. Therefore, the dosage of Mtx should be limited to use of the lowest effective dose to avoid these adverse effects.

Gastrointestinal Prokinetic 5-HT_4 Agonists; Receptor Selectivity and Benefit-to-Risk Profile

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Background: Selective 5-HT_4 receptor agonists (e.g. TD-5108 and prucalopride) appear to produce more robust gastrointestinal (GI) prokinetic activity in patients with chronic constipation than non-selective agonists (e.g. tegaserod or cisapride). Interaction with non-5-HT_4 receptors may also result in adverse effects; cisapride and tegaserod are associated with cardiac arrhythmias (via hERG potassium channel blockade) and ischemic events, respectively. In this study, the pharmacological profiles of several 5-HT_4 receptor agonists are compared.
Methods: Recombinant receptor binding affinities were measured by radioligand techniques. Smooth muscle mechanical activity was studied using guinea pig colon mounted in tissue baths. Colonic transit was measured in conscious guinea pigs (excretion time of dye injected into the colon) as was GI contractility in conscious dogs (implanted with strain gauges).
Results: TD-5108, TD-8954, tegaserod and cisapride had high affinity for the human 5-$HT_{4(c)}$ receptor (mean pK_i = 7.7, 9.4, 8.4 and 7.1, respectively). TD-5108 and TD-8954 had high selectivity for the 5-$HT_{4(c)}$ receptor over all other 5-HT receptor types (>500- and >2,000-fold, respectively). Tegaserod and cisapride had affinity for other 5-HT receptors (mean pK_i = 7.5, 8.4, 7.0, 7.2 and 7.2 for tegaserod at human 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_7 and bovine 5-HT_{1D} receptors, and 6.2 and 7.4 for cisapride at 5-HT_{2A} and 5-HT_{2B} receptors, respectively). TD-5108, TD-8954, tegaserod and cisapride contracted the guinea pig colon (pEC_{50} = 7.9, 8.6, 7.7 and 7.0, respectively); mean intrinsic activities (relative to 5-HT) were 81%, 50%, 47% and 75%, respectively). TD-5108, TD-8954, tegaserod and cisapride (0.03-3 mg/kg sc), increased guinea pig colonic transit; TD-5108 and TD-8954 had the highest potency. In dogs, po dosing with TD-5108 and TD-8954 increased antral, duodenal and jejunal contractility more potently than tegaserod.
Conclusions: There remains an unmet medical need for agents to treat patients with GI disorders such as chronic constipation and constipation-predominant irritable bowel syndrome. Future clinical evaluation with selective 5-HT_4 receptor agonists such as TD-5108 and TD-8954 should determine whether they are associated with a more acceptable benefit-to-risk profile than older, less selective agents.

Gut Health-Promoting Adhesion Of Enteropathogens To Dietary Polysaccharides

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Background: Bacterial adherence to host tissues is regarded as an important initial step for colonisation and infection. Hence, agents that interfere with the ability of pathogens to adhere to gut cells are promising antidotes. Different plant products and food stabilizers were tested in terms of their binding capacity for enteropathogenic bacteria using a miniaturised adhesion test.
Method: Bacterial strains were allowed to adhere to fibrous substances (Table 1) supplied as well coatings in microplates (Becker et al., 2007). Adhering bacteria were provided with liquid medium and allowed to grow during incubation in a microplate reader at 37°C. Bacterial growth was recorded as optical density (OD) at 650 nm over time. The more cells were retained, the shorter the detection time of their subsequent growth. Detection times of growth were determined at an OD of 0.05 ($t_{OD=0.05}$) in triplicate in two independent assays each. Means were compared by Fisher's unprotected least significant difference test.
Results: Table 1 shows the performance of various substances in terms of their binding capacity for different *E. coli* and *Salmonella enterica*.

Table 1. Detection times of growth [h] of different *E. coli* and *Salmonella enterica*. Substances with the shortest detection time bound most bacterial cells (Becker and Galletti, 2008).

Substances	*E. coli* K88 CIDC 1000	*E. coli* K99 CIDC 10	*E. coli* ATCC 25922	*S. enterica sv.* Thyp. RIVM 1287	*S. enterica sv.* Thyp. ATCC 13311	*S. enterica sv.* Ent. ATCC 13076
Artichoke pomace	9.51^{c}	7.49^{a}	2.86^{b}	3.68^{b}	3.97^{b}	$4.64^{b,d}$
BSA (reference)	$10.38^{d,e}$	8.70^{c}	5.14^{g}	3.95^{d}	5.33^{f}	4.58^{b}
Carrot pomace	$10.43^{d,e}$	9.13^{d}	3.23^{d}	4.13^{e}	4.48^{e}	4.82^{c-e}
Konjac gum	10.81^{e}	9.19^{d}	2.90^{c}	3.41^{a}	3.56^{a}	3.81^{a}
Locust bean gum	$9.93^{c,d}$	8.63^{c}	3.51^{f}	4.92^{l}	5.40^{f}	5.00^{e-f}
Palm kernel meal	8.72^{b}	8.31^{b}	3.38^{e}	3.34^{a}	3.79^{b}	$4.63^{b,c}$
Pumpkin pulp and peel	7.48^{a}	8.45^{c}	$2.82^{a,b}$	3.63^{b}	4.24^{c}	5.13^{f-g}
Sesame seed meal	7.74^{a}	8.19^{b}	2.91^{c}	3.40^{a}	3.80^{b}	4.57^{b}
Tempeh (ferm. soya bean)	7.20^{a}	8.56^{c}	2.76^{a}	3.57^{b}	4.18^{c}	4.88^{d-e}
Tomato fruit	7.79^{a}	8.25^{b}	$2.78^{a,b}$	3.68^{b}	4.24^{c}	5.28^{g}

Conclusion: With growth as measuring variable for adhesion, a simple high-throughput method was developed and applied for the screening of large numbers of different food and feed components and bacteria.
References: Becker PM et al. (2007) J. Appl. Microbiol. 103, 2686-2696.
Becker PM, Galletti S (2008) J. Sci. Food Agricult. 88, 2026-2035.
Authors' acknowledgements: This study was funded by the Dutch Product Board of Animal Feed (PDV) and by SAFEWASTES (EU project no. 513949) in equal shares.

Effect of Inoculum Size on the Antibiotic Susceptibilities of Enterobacteriaceae Producing Shv and Ctx-M Extended-Spectrum Beta-Lactamases

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Background and aim: Many extended-spectrum β-lactamases (ESBL) producing isolates of *E. coli* and *K. pneumoniae* are susceptible in vitro to amoxycillin-clavulanate (AMC), ceftazidime-clavulanate (CAZ/cl), and piperacillin-tazobactam (TZP) but MICs increase substantially when higher inoculum is applied. The aim of this study was to determine the effect of inoculum size on the susceptibility of *E. coli* and *K. pneumoniae* isolates with well characterized ESBLs to amoxycillin (AMX), AMC, ceftazidime (CAZ), CAZ/cl, piperacillin (PIP), TZP, imipenem (IMI) and meropenem (MEM).
Material and methods: Minimum inhibitory concentrations (MIC) were determined by broth microdilution method using inocula that differed 100 fold in density according to CLSI. The inocula contained 10^5 CFU/ml and 10^7CFU/ml approximately. The study was performed on the set of *K. pneumoniae* and *E. coli* strains producing SHV-2, SHV-5, SHV-12, CTX-M-3 and CTX-M-15 β-lactamases.
Results: Inoculum effect for CAZ/cl was detected in 52% of SHV-2 producing *K. pneumoniae* strains followed by AMC (43%) and TZP (38%). SHV-5 producing *K. pneumoniae* strains showed the most pronounced inoculum effect with CAZ/cl (57%) and AMC (55%) and to lesser extent with TZP (44%). Inoculum effect was observed for AMC, CAZ/cl and TZP in 71% of SHV-12 producers. *E. coli* producing SHV-5 β-lactamase showed the most pronounced inoculum effect with AMC (61%) followed by CAZ/cl (51%) and TZP (22%). Strains producing CTX-M β-lactamases had a marked inoculum effect with CAZ/cl (71%), AMC (57%) and TZP (50%). AMC and CAZ/cl were associated with inoculum effect against all type of ESBL producers: SHV-2, SHV-5, SHV-12 and CTX-M. TZP was less affected by the inoculum size then AMC, and CAZ/cl particulary with CTX-M producers. The activity of TZP was mostly compromised in the presence of high density of SHV-5 producing *K. pneumoniae*.
Conclusions: Carbapenems were the most stable compounds to inoculum effect regardless of the type of ESBL.

Synthesis and QSAR Studies of CADA Analogs with CD4 Down-modulating and anti-HIV Activities

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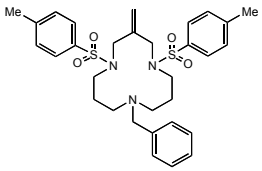

Background: HIV attachment via the CD4 receptor is an important target for developing novel approaches to HIV chemotherapy. Cyclotriazadisulfonamide (CADA, shown at right) inhibits HIV at submicromolar levels by specifically down-modulating cell-surface and intracellular CD4. Aims: 1) To synthesize many CADA analogs having various CD4 down-modulating activities. 2) To develop computational models that can be used to predict activities of novel analogs. 3) To prepare fluorescent analogs for studies in cells.
Methods: The five-step synthesis of CADA was modified to produce more than 50 analogs having various groups on the 3 nitrogen atoms, including some bearing the dansyl fluorophore. Testing 30 of these analogs in MT-4 cells showed a wide range of CD4 down-modulating potencies. Three-dimensional quantitative structure-activity relationship (3D-QSAR) models were constructed using the comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) approaches. To generate these computer models, the solid-state structure of CADA was energy minimized and used to generate the structures of the other 29 analogs.
Results: A CoMFA model was generated having strong statistical significance (r^2 = 0.80, P < 0.001) and internal predictive ability (q^2 = 0.41). Potencies of all 30 compounds are predicted well by this model (within 10-fold of the experimental values). The steric and electrosataic contributions to the model are 59% and 41%, respectively, indicating that the sizes of groups attached to the nitrogens are more important than their polarities. The CoMSIA model also showed the strongest correlation between potency and steric bulk. One of the CADA analogs bearing a dansyl fluorophore is as potent as CADA and has proven to be useful for monitoring uptake and distribution of the drug in T-cells.
Conclusions: 1) Effective approaches have been developed for the synthesis of many CADA analogs with a wide range of potencies. 2) Computer models have been developed for predicting potencies of novel analogs. 3) Fluorescent, potent CADA analogs have been developed that may be useful for elucidating the mechanism by which CADA compounds down-modulate CD4.

Pharmacokinetics and Pharmacodynamics of Amphotericin B and its Lipid Formulations

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Background: Because of its broad spectrum, the polyene amphotericin B (AMB) has still an important role in treatment of invasive fungal infections. It is eliminated unchanged via the bile and the urine. However, its use is limited by infusion related adverse events and by its nephro-toxicity. Three less toxic lipid formulations are available, displaying different composition of their lipid moieties and thus different particle size and shape. Liposomal AMB (LAMB) comprises small uni-laminar vesicles. Its volume of distributions is small, its half-life is short and high plasma concentrations are achieved. AMB lipid complex (ABLC) forms large ribbon-like structures resulting in a large volume of distribution a half-life of about 1 week whereas only low plasma level are reached. The 3rd lipid formulation, AMB colloidal dispersion (ABCD) displays intermediate plasma levels, half-life and volume of distribution.
Methods: AMB pharmacokinetics was analyzed in 18 critically ill patients on treatment with lipid-formulated AMB (13 requiring hemofiltration). Lipid-associated and liberated AMB were separated by solid phase extraction and quantified by high performance liquid chromatography. AMB tissue distribution was assessed in autopsy sample obtained from 32 patients who had died during therapy with lipid formulated AMB.
Results: In critically patients, mean peak plasma levels (Cmax) of liberated AMB amounted to 0.5 µg/ml after infusion of LAMB or ABCD, whereas Cmax of total AMB was 3.4 µg/ml and 0.8 µg after ABCD. The clearance (CL) of liberated AMB was 0.2 l/kg/h (CL of LAMB [lipid-formulated] = 0.08 l/kg/h, CL of ABCD = 3.0 l/kg/h). AMB-pharmacokinetics on and off hemofiltration were similar.
Tissue concentrations of 100 µg/g were reached in the liver, 70µg/g in the spleen. Lung concentrations were significantly higher after ABCD than after LAMB treatment (33 vs.12 µg/g). Levels in myocardium and brain were 3 µg/g and 1 µg/g, respectively.
Conclusions: 1) The lipid-associated moieties of AMB lipid formulations display considerable differences in their pharmacokinetics whereas pharmacokinetics of the liberated AMB fraction is independent from the preparation administered. 2) No dose adjustment is required during hemofiltration. 3) AMB accumulates in liver and spleen, tissue concentrations in lung and kidneys are intermediate and low in myocardium and brain.

Mitochondrial Respiratory Chain (MRC) and Mitochondrial Permeability Transition (MPT) Effectors against Heavy Metal-induced Cytotoxicity: State-of-the-art and Perspective

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Background: Heavy metals, such as cadmium, mercury, etc. are strong carcinogenic, genotoxic and cytotoxic agents of high environmental and occupational hazard. Mitochondria are important targets for these pollutants. MRC disturbance and activation of different ionic channels in inner mitochondrial membrane, mainly nonselective MPT pore, are considered to be the key events in different types of cell death. Aims: 1) To find effective protectors against the heavy metal-induced cytotoxity.
Methods: To further underscore mechanism(s) and role of mitochondrial dysfunction in toxic action of heavy metals we used rat neuronal cell line PC12, rat ascites hepatoma AS-30D cells and isolated rat liver mitochondria as a model system, as well as flow cytometry, different fluorescent probes and ion-selective electrodes, and swelling technique.
Results: We found that against Cd^{2+}-induced injury not only well-known antioxidants, such as N-acetylcysteine, vitamin E, butylhydroxytoluene, and MPT inhibitors – cyclosporine A, bongkrekic acid, ADP, Mg^{2+} and dithiothreitol are effective but also inhibitors of different MRC components, namely of complex I (rotenone), and of complex III (stigmatellin and antimycin A). Interestingly, stigmatellin was shown to be one of the strongest protectors that exhibited its action not only on isolated mitochondria but on both types of the cells.
Conclusions: 1) Our data pointing to the direct involvement of the MRC, and especially reactive oxygen species (ROS) production on respiratory complex III in Cd^{2+}-induced mitochondrial membrane permeabilization and cell death. 2) On the basis of own findings and data in literature, we proposed a hypothetical model of MPT pore structure and/or regulation with the involvement of a mitochondrial supercomplex formed by respiratory complex I (P-site) and III (S-site) which may constitute not only critical Me^{2+}-binding sites but also main loci for ROS generation that was instrumental in oxidation of thiol groups crucial for MPT pore induction; moreover, depending on conditions and cell type, either one or both respiratory complexes could participate in triggering of the MPT pore assembly and cell death. 3) The data obtained are important for searching new drugs which could become real "magic bullets" in the nearest future.
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All abstracts are listed in alphabetical order of the presenting author.

Susceptibilities of rickettsiae to antimicrobials.

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Abstract:
Rickettsiae grow only intracellularly. The antibiotic susceptibility is assessed by plaque,dye uptake or IF assays. Rickettsiae are susceptible to doxycycline, thiamphenicol and fluroquinolones. Betalactams, aminoglycosides and cotrimoxazole are not active. Typhus group rickettsiae are susceptible to all macrolides,whereas the spotted fever are more resistant to rifampicin than the other rickettsiae. Rickettsiae felis is not susceptible to gentamicin, erythromycine, amoxicilline or cotrimoxazole. we present an overview of susceptibility of rickettsiae to antimicrobials.

Immunogenicity of Biopharmaceuticals - An Inconvenient Truth.

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Background: Today, recombinant gene technology permits the use of drugs which are almost identical with natural human proteins, including antibodies (Abs). Many assume that these drugs pose little or no risk of triggering specific immune responses, because patients according to dogma are tolerant towards their own proteins. Unfortunately, this is not the case, and even so-called 100% human biologicals may be immunogenic. I shall focus on the immunogenicity of anti-TNF Ab constructs plus the largely ignored problem of individual variations in drug bioavailability (BA) and pharmacokinetics (PK).
Methods: Several methods have been used to assess circulating levels of anti-TNF biologicals as well as Abs to these drugs. Most are based on solid-phase technology, *e.g.* ELISA, with their inherent problems of low sensitivity, false positivity and susceptibility to nonspecific interference, fx by rheumatoid factors. We have developed fluid-phase radioimmunoassays (RIAs) for monitoring patients on anti-TNF biologicals, one for functional blood levels of the drugs, and one for anti-drug Abs.
Results: We measured BA/PK and anti-drug Abs developed in 'anti-TNF-immunized' patients with rheumatoid arthritis; most of these were treated with infliximab. The most sensitive anti-drug Ab assay involved binding to soluble and intact infliximab rather than to plastic-immobilized drug. Indeed, data obtained by solid-phase assay using cross-binding of plastic-fixed and soluble infliximab were inconsistent with results obtained with fluid-phase RIA. Despite intravenous administration, there were sizable interindividual variations in serum trough levels of the drugs even at time-points where anti-drug Abs had not yet developed; these levels diminished or disappeared in parallel with Ab induction (30% and 44% of patients on infliximab were Ab-positive at 3 and 6 months, respectively). Abs were 'neutralizing' in that their levels were positively associated with inhibition of TNF binding to the drugs. There were highly significant correlations between high levels of anti-drug Abs and later dose increases, side-effects and cessation of therapy.
Conclusions: To prevent prolonged use of inadequate anti-TNF biotherapies, individualized assessments of BA/PK and Abs seem essential (personalized medicine). In our hands, fluid-phase assays are superior to solid-phase assays.

The Path from Colles' Law to the "Magic Bullet"

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The hypothesis that syphilis might be counteracted immunologically can be traced to the Irish surgeon, Abraham Colles, who noted in 1837 that a congenitally syphilitic infant may be born of a mother who showed no signs of syphilis. He interpreted this to indicate that the infant, infected by a diseased fertilizing sperm, immunizes its mother against syphilis, even though it can infect other caretakers. This talk will sketch the disappointing investigations that led from "Colles' law" to the reception of Ehrlich's "magic bullet."

The first line of serologic investigation, initiated by Joseph Auziaz-Turenne in the 1840s was called "syphilization." In this controversial therapy the patient was "saturated" over several months with hundreds of subcutaneous injections of "syphilitic toxin," based on the hypothesis that when saturation was reached new manifestations of the disease were prevented, after which cure might be achieved. Saturation had been achieved when injections no longer elicited a local inflammatory reaction. Syphilis and chancroid had not been differentiated at this time. Thus the misinterpretation of "saturation" of syphilitic patients was frequently compounded by the injection of chancroidal rather than syphilitic serum. Most responsible for perpetuating this technique until about 1870 was the Norwegian venereologist, Caesar Boeck.

With the advent of bacteriology it became of interest why certain species were resistant to a pathogen that was lethal to another. Could this "resistance factor" be transferred, either as prophylaxis or therapy? The first injections of serum from animals that could not be made syphilitic were undertaken in 1890 on patients with secondary syphilis and were recognized to be ineffective. Further trials were stimulated by favorable reports by an Italian venereologist who in 1892 gave subcutaneous injections of lambs' blood. Various investigators used serum from dogs, rabbits, sheep, and horses. When the use of serum from untreated animals became recognized as useless, donor animals were pre-treated with syphilitic "toxin" or mercury. The greatest stimulus to experimentation with serum therapy came in 1893 with the announcement of successful serum treatment of diphtheria and tetanus. The lack of a proven pathogen of syphilis added confusion to the search for an anti-syphilitic serum.

The immediate acceptance of the discovery in 1905 of the *Spirochaeta pallida* as the pathogen, rapidly followed by discovery of a practical diagnostic method, may explain why, in the midst of the belief that a curative serum for all microbial diseases was about to be discovered, Ehrlich's announcement in 1910 of the therapeutic efficacy of medicinal "Salvarsan" was received so enthusiastically. Ehrlich was more circumspect regarding the usefulness of Salvarsan than many of its early advocates.

Water Channel Proteins (Aquaporins): From their Discovery in 1985 in Cluj-Napoca, Romania (By the use of a Doping Nmr Method and Specific Labeling) to the use of their Inhibitors as Magic Bullets

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Water channels or water channel proteins (WCPs) are transmembrane proteins that have a specific three-dimensional structure with a pore that can be permeated by water molecules. The first WCP was discovered in the human red blood cell membrane in Cluj-Napoca, Romania, in 1985 by Benga's group. We have measured the water permeability of human red blood cells (RBCs) by a doping NMR method. We showed for the first time by NMR that the parameters characterizing diffusional water permeability are the same in RBCs and resealed ghosts and reported the largest series of determinations of water diffusional permeability of RBCs available in literature.
The first water channel protein (WCP), later called aquaporin 1 (AQP1) was discovered in the RBC membrane by my group in 1985 in Cluj-Napoca, Romania, reported in publications in 1986 (Benga et al., *Biochemistry*, **25**, 1535-1538, 1986; Benga et al., *Eur. J. Cell Biol.*, **41**, 252-262, 1986) and reviewed in the following years. This discovery was achieved by specific labelling of the RBC membranes with the known water transport inhibitor ^{203}Hg - *p*-chloromercuribenzenesulfonate (PCMBS). In parallel, the water permeability was measured by the doping NMR technique and the inhibition induced by PCMBS was calculated. The priority of Benga in the discovery of the first WCP was acknowledged by many outstanding scientists.
We also have a world priority in the discovery of the implications of water channel proteins in epilepsy (Benga and Morariu, Nature**,** **265**, 636-638, 1977) and Duchenne muscular dystrophy (Serbu et al., Muscle & Nerve, **9**, 243-247, 1986). These findings were interpreted as an expression of generalized membrane defects affecting water permeability in epilepsy and Duchenne muscular dystrophy. In recent years this idea was confirmed.
Since the discovery of WCPs tremendous progress in understanding their role in physiology and pathology. Based on these advances it became clear that inhbitors of AQPs can be used as magic bullets in a variety of diseases, including cancer. The doping NMR method should be used to compare the effects of inhibitors of WCPs as magic bullets. Examples of this approach will be given.

A Study of Capecitabine and Cisplatin in the Treatment of Recurrent Carcinoma of the Uterine Cervix

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Background: Platinum is the mainstay of treatment in advanced or recurrent cervical carcinoma, however, the duration of response is short lived as well as the median survival. Fluorouracil (5-FU) has been shown to be active in cervical carcinoma. Capecitabine, an oral fluoropyrimidine carbamate, is sequentially converted to 5-FU by thymidine phosphorylase (TP) which is found at higher concentrations in cervical carcinoma than normal tissue. In addition, cisplatin further upregulates TP. Capecitabine plus cisplatin has the potential to be an active treatment, which is more convenient than 5-FU-cisplatin.
Methods: This study combines capecitabine and platinum in patients with recurrent cervical carcinoma with no potentially curative standard treatments. Sixteen patients (14 squamous cell carcinoma, and 2 adenocarcinoma) with a median disease-free interval of 11 months (range, 2-96) received cisplatin 50 mg/m^2 intravenously on day 1 and oral capecitabine 1000 mg/m^2 twice daily for two weeks with a one week rest period.
Results: Median age was 50 years (range, 31-74). A total of 89 cycles were administered with a mean of 5.5 cycles (range, 3-6) per patient. Four of the sixteen patients had complete response (25%), 4 had partial response (25%), and 5 had disease stabilization (31%). Ten patients (63%) had recurrent disease outside the radiation field. The overall response rate in patients with recurrent disease within the previous irradiated field was 33% and 60% in patients with tumor outside the irradiated field. The median follow-up time was 29 months (range, 11-39). The median time to progression was 9 months (range, 5-37), with a median overall survival of 23 months (range, 5-37). The majority of adverse events were mild and there were no grade 4 adverse events. Hematological toxicity was the most frequent adverse event with grade 3 neutropenia in 19% of patients. Grade 2 and 3 hand-foot syndrome occurred in 38% and 6% of patients, respectively. There were no chemotherapy-related deaths.
Conclusions: This active yet convenient combination of capecitabine and cisplatin shows a high response rate, long time to progression and survival with acceptable toxicities for patients with recurrent carcinoma of uterine cervix.

Girolline, a new antiplasmodial leader extracted from the sponge *Cymbastela cantharella*

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Background: Malaria is the most prevalent parasitic disease in the world today. In this context, there is clear demand to search for new antimalarial agents and research into new antimalarial drug candidates originating from natural sources has been actively pursued. Girolline (Figure1), a 2-aminoimidazole derivative extracted from *Cymbastela cantharella* (a New-Caledonian Sponge) already known for its antitumor activity, was tested against *Plasmodium*.
Methods: We evaluated the effects of girolline and some of its analogues *in vitro* and *in vivo* against *P. falciparum* and *P. vinckei petteri*, respectively. We then evaluated its toxicity *in vitro* and *in vivo*. We have also determined the point of action of girolline in the erythrocytic life cycle of the malarial parasite and its synergistic action with chloroquine.
Results: We have demonstrated that girolline presents a very promising activity against malaria both *in vitro* against four *P. falciparum* strains and *in vivo* on murine mdel. Girolline also showed a specific mode of action by inhibiting *Plasmodium* protein synthesis. Moreover, between girolline and chloroquine, a high synergistic effect was reported.
Conclusions: Girolline is of a real interest as research basis for a new class of antimalarials. With such a biological profile, girolline could be considered as a model chemical structure for new candidates in the arsenal of new drugs and in particular of drugs able to fight malaria.

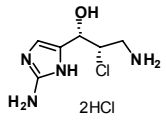

Figure 1: Structure of Girolline

Natural Products (NP) - Microbial Metabolites (MM) - Antibiotics (AB): History, Facts and Problems, Where Now ?

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History: Great benefit and unpredictable negative consequences. The NP research and especially the discovery of new MM declined in the past years. Few new drugs were discovered. The reasons are: resistance-problem, less success with HTS and combinatorial chemistry, but the reasons are mainly economic and regulatory. In the last years the total synthetic efforts and the discovery of compounds from higher plants and marine organisms increasing, but sometimes no evident proof to the real producer species (endophytes, symbionts - taxol, patellamide).
Facts: Close to half a million NP, including ~100 000 MM, ~20 000 microbial AB (~350 marketed), there are known. High percentage, (~40 %) of all drugs and about 70-80 % of all known AB drugs were derived from NP, as direct drugs, derivatives (semisynthetic and modified products), and other NP mimics (synthesized as NP analogue). The NP libraries have some advantages over random synthetic or combinatorial chemical libraries in several respects (e.g. complex structures hardly accessible by chemical methods). "Nature is the best combinatiorial chemist". They also meet the green chemistry. The bioproducts have inherent - but perhaps undiscovered - biological functions and drug-like structures, compatible to the host. The real function of MM is the communication with other microbes, higher organisms and the environment. They are the interface between microbes and the rest of world. The biosynthetic pathway of microbes, evolved in the millions of years under evolutionary pressure, ares mainly undiscovered (silent genes). Only ~1% of the existing bacteria are cultivable. Unlimited number of possible new structures exists in coded form in the metagenom.
Where Now: Presently everything seems to be out of balance. Back to the pre-antibiotic era ? What can we do ?
1. Diversification of NP libraries. New ways to discover new leads to medicinal chemistry (inspiration for synthetic chemists). 2. New biochemical/genetic methods: mining and engineering of the biosynthesis (genom). 3. Discover new sources (marine species, endophytes, uncultivable, rare microbes). 4. New target-oriented effective screenings. 5.Chemical and biosynthetic post-evolution by combinatorial methods. 6. Better understanding and solving the problems of the drug-target-host interactions. The key of our co-evolution with microbes is the exact understanding of the life-cycle of microbes.

Possible Consequences of Transplacental Transfer of Viruses in Healthy Pregnants (Rewiev)

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Background: Viruses, which may cause illnesses of the fetus were shown to be transfered frequently through the placenta into fetal tissues without any clinical consequences. Rubella and rubella vaccine, measles and measles vaccine, Togaviruses, Flaviviruses, hepacivirus (HCV), Hepadnaviruses (HBV), , human cytomegalovirus (HCMV), human herpesvirus types 1 (HSV), 6 (HHV6), 7 (HHV7), and 8 (HHV8), human parvovirus B19 (HPV-B19), dependovirus (AAV), human adenovirus, Epstein-Barr virus (EBV), human papillomaviruses (HPaV), human polyomaviruses (HPyV), lentiviruses and the Anellovirus TTV were detected in the umbilical blood, amniotic fluid or fetal tissues at the end of healthy pregnancies.
Mechanisms: IgG transport mechanisms, transported maternal cells and the lipid rafts were shown to be vehicles of this virus transport.
Results:
1.) The first consequence of the contact of the developping fetal organism with latent viruses can be the life-long carriage of these viruses upon birth.
2.) The developping fetal immune system might create immunotolerance to certain viral antigens depending on the fact, whether the viruses replicate or are only latently present in the fetal cells. This partial immune tolerance may impair the post partum immune response facilitating tumour formation in the affected individuals.
3.) Herpesviruses may activate endogeneous retroviral genes in fetal cells, modifying their differentiation and surface properties. Alternatively the pathogenesis of autoimmune diseases might be initiated by these modifications.
4.) The viruses are coding for micro RNA molecules possibly influencing the replication and differentiation of the virus carrier cells. The genes of DNA viruses interfering with apoptotic mechanisms may also disturb the normal differentiation processes in the organs of the fetus.
5.) Finally the response to the mandatory antiviral vaccinations might be impaired by the transplacentally transcytosed viruses.
Conclusion: The systematic testing of umbilical blood and urine of newborn babies would be of essential importance using molecular technics for the presence of the viruses in order to be prepared for yet unknown risks for the future life span.

Lacosamide is a Novel Antinociceptive and Antiepileptic Drug with a Dual Mode of Action

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Background: Lacosamide is an investigational drug that has demonstrated positive results in Phase III trials of neuropathic pain and epilepsy. Preclinical studies have shown neuroprotective effects of lacosamide both in animal models and in-vitro. Electrophysiology and proteomics experiments have identified two likely modes of action for lacosamide.

Methods and Results: Electrophysiology experiments performed in mouse neuroblastoma cells indicate that lacosamide reduces sodium-channel availability by selectively enhancing slow-inactivation. Enhancing slow-inactivation is thought to raise channel-activation thresholds, reducing pathophysiological neuronal hyperexcitability. This mechanism is different from that of anesthetic and other antiepileptic agents, which non-selectively block the sodium channel pore and/or enhance fast- and slow-channel inactivation.

A second mechanism of action may occur via the binding of lacosamide to collapsin-response mediator protein 2 (CRMP-2), a phosphoprotein that is involved in neuronal differentiation and axonal out-growth (processes that are maladaptive in the pathophysiology of pain and epilepsy). The interaction of lacosamide with CRMP-2 may underlie the apparent neuroprotective effects of lacosamide, since CRMP-2 appears to be important for mediating neuroprotection from excitotoxic insult and apoptosis.

Conclusions: The dual mode of action of lacosamide represents two novel mechanisms for the treatment of neuropathic pain and epilepsy. Based on current studies, it is proposed that selective enhancement of slow-inactivation of sodium channels may underlie the immediate effects of lacosamide. Further characterization of the interaction with CRMP-2 may help to explain its role in lacosamide's symptomatic and disease-modifying effects.

PK/PD Relationship of Antibiotics in Local Treatment of Prostethic Infections

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Background. Infection is the most serious complication following orthopaedic surgery, and delivery of antibiotics to the surgical area is a way of reducing the infection frequency.
Polymethylmethacrylate (PMMA) cements impregnated with antibiotics are currently utilised as local antibiotic carrier in orthopaedic surgical-site infection to treat prosthetic infections (hip, knee, etc) and are an adjunct to current therapy (surgical debridment and systemic antibiotic therapy).
Cement intrinsic characteristics and capacity to release drug are essential for the final clinical outcome as well as antimicrobial drug pharmacodynamics at the site of infection.
Methods. Several experimental models *in vitro* and *in vivo* have been developed to better understand the release kinetic of different antibiotic from cement and to optimise their use in clinical practice.
Results. Aminoglycosides (A) and vancomycin (V) show good positive characteristics: bactericidal activity, adequate release, compatibility, mechanical resistance, and excellent tolerability.
The release of A and V from PMMA cement seems prompt and effective, determining high local concentrations. The drug elution shows a bimodal profile, consisting of an initial high rapid release of drug followed by a much slower but sustained release. Initial drug concentration, cement surface area and porosity are important factors in determining the amount of drug release.
A and V in combination show synergistic antimicrobial activity both *in vitro* and *in vivo* against multiresistant clinical isolates.
A and V concentrations in drainage fluid following spacer implant are higher than those obtained with systemic administration; their kinetics is superimposable. These high local concentrations of the combination are effective against multiresistant pathogens responsible for prosthetic infections (high inhibitory bactericidal titre of drainage fluids).
Moreover, the presence of A and V in PMMA specimens reduces the growth and bacterial adhesion of susceptible and intermediate-resistant Staphylococci. Their anti-adhesive effect depends on the characteristics of the microorganism and its capacity of adhering to antibiotic-loaded surfaces.
Conclusions. PMMA antibiotic loaded cements improve local drug delivery at infection site and enhance pharmacodynamics of antibiotics for treatment of prosthetic orthopaedic infections.

Development of Unique Cisplatin Analogs for Site-Specific Treatment of Hormone-Dependent Female Cancers

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Background: Chemotherapy remains, to this day, an effective treatment for several types of cancer. However, the severe side effects caused by the treatment limits its full potential for a cure. Thus, the development of site-specific anticancer therapy is a subject of intence research. Several strategies can be used to target cancer cells. For instance, the use of a carrier molecule being able to recongnise a specific receptor in the cell is a tactic of choice used by several research groups. We have developed several estradiol-platinum(II) (E_2-Pt(II)) hybrid molecules using the following guiding principles a) potential for affinity towards the estrogen receptor b) potential for *in vitro* and *in vivo* selectivity on hormone-dependent female cancers c) ease of synthesis and, d) potential for large scale industrial production.
Methods: The development of the E_2-Pt(II) hybrid molecules will be initially discussed. Then, the most promising hybrid derivative, VP-128, is selected to examine its biological activity towards breast (MCF-7, ZR-75-1, MDA-MB-468, MDA-MB-231 and HS578-T) and ovarian (OVCAR-3, SKOV-3, A2780 and A2780-cp) cancer cells, *in vitro* (MTT assays) and *in vivo* (xenografts model) using ERα positive or negative cells.
Results: MTT assays revealed that VP-128 decreased the viability of breast and ovarian cancer cells more efficiently than cisplatin itself *in vitro*. Moreover, in the case of breast cancer the expression of ERα sensitized the cells to the growth-suppressive effect of VP-128. Hoescht nuclear staining revealed an improved efficiency of VP-128 compared to cisplatin to induce apoptosis of breast cancer cells, which was enhanced in ERα-positive cells. In cisplatin resistant A2780-cp cells, VP-128 was able to induce cell death indicating that the new drug might also be efficient to kill cisplatin resistant cancers. Finally, using human breast and ovarian cancer cell xenografts in nude mice, we found that VP-128 had stronger antitumour activity compared to cisplatin *in vivo*, and was more specific and selective towards hormone-dependent cancer cells.
Conclusions: Experimental data show that VP-128 possesses enhanced anticancer activity compared to cisplatin and is able to specifically target hormone-dependent tumours in an *in vivo* model. Thus ultimately, VP-128 could provide new and/or alternative treatment modalities for breast and ovarian cancers.

Mediterranean spotted fever(MSF) in Oran (Algeria)

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Abstract
MSF due to rickettsia conori was thought ,for many years,to be the only tick-borne rickettsial disease prevalent in Algeria. However ,in recent years,other species within the spotted fever group of the genus Rickettsia have been described as emrging pathogens. Tick-borne agents include:Rickettsia aeschlimanii and rickettsia massiliae. Many rickettsia of unknown pathogenicity have also been detected from ticks and could represent potential emerging pathogens to be discovered in the future. Furthermore,a new spotted fever rickettsia,Rickettsia felis ,was found to be associated with cat flees and is an emerging human pathogen. Rickettsia felis is susceptible to doxycyclin,thiamphenicol,and fluoroquinolons but not to gentamicin,erythromycin ,amoxicillin or trimethoprim-sulfamethoxazole. The resistance of this new species to erythromycin is consistent with taxonomic position within the spotted fever group. We present an overview of these rickettsiodes,focusing on emerging diseases.

New Approaches for the Use of Old Drugs – Preclinical and Clinical Pharmacological Evaluation of the Pyrimidine Anti-neoplastics: 5-fluorouracil, zebularine, 5-fluoro-2'-deoxycytidine, gemcitabine, and THU.

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Background: Pyrimidines include some of the oldest anti-neoplastic agents. They are still an important part of many anticancer drug regimens, and continue to generate interest, as evidenced by recent studies of zebularine and 5-fluoro-2'-deoxycytidine (FdC), two DNA hypomethylating agents. Our aim is to develop innovative pyrimidine anticancer drugs and improve the use of existing ones.
Methods: We developed chromatographic assays for zebularine and 3 metabolites (HPLC-radiodetection), FdC and 4 metabolites, gemcitabine (dFdC) and metabolite, 5-fluorouracil (5-FU), and tetrahydrouridine (THU) (all LC-MS/MS), and applied these to preclinical and clinical pharmacological studies.
Results: Preclinical studies revealed that, *in vivo*, [^{14}C]-zebularine is quickly converted to uridine, producing hydrogen peroxide as a by-product. In mice, THU decreased metabolic degradation of FdC (>50-fold) and dFdC (5-fold) and substantially increased their oral bioavailability (25-fold and 4-fold, respectively). Clinical pharmacokinetics of intravenous FdC and THU were determined, and the oral route is being explored. Preclinical studies show that oral dFdC and THU is efficacious in a CFPAC-1 xenograft pancreatic tumor model (Treated/Control = 30%). The 5-FU LC-MS/MS assay was shown to correlate well with a newly developed 5-FU immunoassay, which will allow on-demand therapeutic drug monitoring.
Conclusions: Pyrimidines are an important class of anticancer drugs, both in established regimens, and as agents in future treatments. Their metabolism is complex, but can be modulated to allow oral dosing, and different dosing schedules. New applications and targets of pyrimidine drugs are being discovered and should maintain interest in this class of compounds.

Novel Diastereoselective Synthetic Routes to Tamoxifen, Toremifene, and Droloxifene, Anti-Breast Cancer Agents *via* Organoboranes

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Background: Tamoxifen., toremifene, and drroloxifene are useful drugs used in the treatment of breast cancer. Several synthetic methods are known in literature to synthesize them. Some of the methods give a mixture of diastereomers. Organoborane reagents are known to achieve high selectivity in organic synthesis. Consequently, highly diastereoselective synthesis of tamoxifen, toremifene, and droloxifene is achieved using organoborane reagents. Our current methods are compared with the literature methods to prepare these drugs.
Methods: This study included the reactions of alkynylborates with an electrophile such as trimethylchlorostannane followed by stepwise Stille coupling and Suzuki coupling. Since the chemicals used are highly air-sensitive, all of the reactions are carried out under nitrogen atmosphere. In the case of tamoxifen synthesis, phenylethynyllithium is reacted with triethylborane followed by Stille coupling with (*N*-{2-(4-bromophenoxy)ethyl}*N,N*-dimethylamine and Suzuki coupling with bromobenzene in the presence of tetrakis(triphenylphosphine)palladium. In the case of toremifene, 4-chloro-1-butynyllithium is reacted with triphenylborane followed by reaction with trimethylchlorostannane. The resulting intermediate is then subjected to palladium catalyzed Stille coupling with bromobenzene and palladium catalyzed Suzuki coupling with (*N*-{2-(4-bromophenoxy)ethyl}*N,N*-dimethylamine In a similar manner, droloxifene is synthesized by the reaction of 4-[2-(dimethylamino)ethoxy}-phenylethynyllithium with triphenylborane and chlorotrimethylstannane. The resulting intermediate is subjected to palladium catalyzed Stille and Suzuki coupling reactions with *m*-bromophenol and ethylbromide respectively.
Results: Tamoxifen is obtained in 55% isolated yield. Toremifene and droloxifene are obtained in 48% and 50% isolated yields respectively. These compounds are purified by column chromatography.. The structures of these drugs are confirmed by nuclear magnetic resonance (NMR) spectroscopic methods.
Conclusions: In summation, highly diastreoselective synthesis of tamoxifen, toremifene, and droloxifene is achieved using organoborane reagents, Stille, and Suzuki coupling reactions. The optimization of reaction yields of these drugs is currently in progress in our laboratory.

Mechanisms that Explain the Lower Incidence of Breast Cancer in Postmenopausal Women Treated with Conjugated Estrogens (CEE). Role of Estrogen Receptor α and β.

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Background: Recent findings from the Women's Health Estrogen Alone Trial (JAMA 2008), showed that long term treatment of hysterectomized postmenopausal women with CEE not only did not increase the incidence of breast cancer, but more importantly, may have reduced the risk in these women for this disease. These results raise some important questions as to the mechanisms involved and whether all types and of estrogen can impart this protection. In the present study, the relative binding affinities (RBAs) of 11 equine estrogens and their functional activities mediated via ERα and ERβ were compared.
Methods: The RBAs were estimated by competitive binding assays using ^{3}H-17β estradiol and unlabelled equine estrogens. The functional activity of the estrogens was measured in HepG2 cells transfected with human ERα or ERβ or both and secreted-alkaline phosphalase (SEAP) gene and analyzed by a chemiluminescent assay.
Results: In comparison to 17β-estradiol (17β-E_2), the RBA's of most ring B unsaturated estrogens were 2-8 fold lower for ERα and ERβ, however, these unique estrogens had 2-4 times greater affinity for ERβ than for ERα. The transcriptional activity of these 11 estrogens showed that all estrogens were functionally active. 17β-estradiol induced the activity of SEAP by ERα to a higher level than any other estrogen. Activity of other estrogens was 12 to 17% that of 17β-E_2. In contrast, 17β-E_2 stimulated the activity of ERβ to a 5 fold lower level than with ERα. The activities of other estrogens mediated via ERβ were 66-290% that of 17β-E_2, with equilenin being the most active. Except for 17β-E_2, no correlation was observed between functional activities and the RBA's for ERs. The activity of the ring B unsaturated estrogen components of CEE appear to be exerted predominantly through ERβ. To our knowledge, these are first such observations. Moreover, depending on the estrogen, ERβ can act as a dominant repressor or dominant activator of ERα's transcriptional activity.
Conclusions: Taken together, these data indicate that all estrogens are not the same and have different pharmacology. Some ring B unsaturated estrogen components of CEE can via ER β inhibit the proliferative effects of 17β-estradiol mediated via ERα, thereby reduce the risk of breast cancer in women who are just taking equine estrogens alone.

Nanodevices for targeted delivery: An evaluation of toxicological models

BHOGAL N

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Background: Engineered nanoparticles and liposomal delivery systems (nanodevices) are increasingly utilised and investigated as a means of overcoming problems with the bioavailability, stability and toxicity of therapeutics. Such systems promise to revolutionise clinical management and to obviate the need for needle-based self-administration.
Methods: Some of the major challenges are described and the prospects for simplifying and stratifying toxicological assessment of nanodevices is considered. The importance of selecting the most appropriate toxicological models for biocompatibility testing is highlighted by the surge of new nanodevice-medicine combinations currently in the pipeline.
Results: Nanomaterials display unique reactivities that are dependent on physicochemical properties, surface chemistry and biophysical and biological interactions. These reactivities are often very different from those of bulk materials and can be unpredictable owing to material heterogeneity. The estimation of the rate of drug release from nanodelivery devices, issues pertaining to bioretention are problematic.
Differences in anatomical features between test species and human, including accessibility via natural barriers, targeting dependent on physical characteristics of tissue vasculature, immune cell density and lymphatic system characteristics present interesting challenges. For instance, it is clear that transdermal delivery is likely to depend on follicular transport. Yet animal models, with their vastly different follicle diameters are unlikely to give an indication of absorption through human skin. Similarly, it is clear that human skin is much more densely innervated and supplied with immune cells that most species. Thus, the relevance of animal models to assess the biocompatibility and as well bioavailability via this route of delivery is highly questionable. Whether human skin equivalents or cadaveric human skin can successfully replace animal studies is dubious, particular given that most organotypic models lack follicles, paracrine function and full barrier function.
Conclusions: The application of existing animal and *in silico* approaches to resolving species differences and informing biocompatibility testing strategies of nanoparticles is questionable, calling for a review of methods for nanomaterial testing.

Authors' disclosure statement: FRAME is a scientific charity that conducts research into scientifically sound alternatives to animal testing that afford the highest standards of human health protection.

New derivatives of BM 212 with improved antimycobacterial activity.

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Background: According to the report 2007, compiled by the World Health Organization (WHO), the total number of new cases of Tuberculosis (TB) worldwide in 2005 had risen to approximately 8.8 million and there were approximately 1.6 million TB deaths during the same year. Increased infection with the M. avium complex (MAC) is also contributing to the morbidity and mortality in AIDS patients. The most urgent goal of chemotherapy of tuberculosis infections should be the development of highly active and low-cost drugs, which should be used not only in industrialized countries but also in developing ones in which both these infections are now rapidly increasing. As active molecules already introduced in therapy very soon generate resistance, scientists have focused their attention on the development of new antimycobacterial compounds acting with a mode of action without cross-resistance.
Methods: Many pyrrole derivatives, analogues of BM 212, have been synthesized on the basis of previous results and molecular modelling considerations based on the pharmacophore model previously identified for them. All the derivatives were tested for their cytotoxicity and in vitro activity against many strains of *M. tuberculosis*, atypical mycobacteria, drug-resistant mycobacteria of clinical origin and intracellular mycobacteria. Protection Index (PI) was calculated and for the most active of them the bioavailability was also evaluated. The in vivo tests and the study of the mode of action are currently under study.
Results: Some of the synthesized compounds revealed more active than BM 212 against mycobacteria. In particular the PI for many of them was comparable to that of reference compounds, Isoniazid, Streptomycin and Rifampin. Many of the synthesized compounds revealed also to be active against intracellular mycobacteria and they showed to inhibit drug-resistant mycobacteria of clinical origin.
Conclusions: On the basis of our previous studies and molecular modelling considerations, many new derivatives of BM 212 were identified. In particular some of them revealed very active and low toxic so that they can be considered very promising for future studies.

The Insulin-Stem Cell Connection: What Insulin Does To Stem Cells And How We Get It From Them

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Background: Replacement of pancreatic β-cells by stem cell-derived insulin producing cells (IPCs) is a promising strategy to treat diabetes. Therefore, generating IPCs has been a major goal of academic and industrial efforts to harness the differentiation potential of embryonic or adult stem (ES or AS) cells. To date, most differentiation protocols yield only a small proportion (<5%) of cells that genuinely produce sufficient amount of insulin in response to high glucose levels. A major obstacle in identifying true IPCs is that serum-free culture conditions require supplements with insulin to ensure survival of the differentiating stem cells. This exogenous insulin is taken up by the cells and confounds the determination of cell-derived insulin using antibody-based techniques.
Methods: Our laboratory has developed, for the first time, a differentiation protocol replacing insulin with a recombinant peptide analog of insulin-like growth factor-1 (IGF-1). This IGF-1 analog-supplemented, serum-free medium (NeoITS medium) allows for the identification of genuine IPCs.
Results: Using chemically defined NeoITS medium instead of insulin/transferrin/selenite (ITS) or serum supplement, we derived nestin(+)/C-peptide(+) and nestin(-)/C-peptide(+) cells at proportions of 40% or 25%, respectively. These IPCs increased insulin secretion by 20-fold when exposed to 20 mM glucose. The determination of secreted insulin was not possible when ES cells were differentiated in the presence of conventional ITS or serum.
Conclusions: Insulin or IGF-1 are essential cell survival factors for differentiating stem cells. A recombinant peptide analog of IGF-1 in NeoITS can efficiently replace IGF-1 or insulin and allows for the determination of secreted insulin. Using NeoITS will tremendously facilitate the identification of early IPCs when differentiated from ES or AS cells (including induced pluripotent stem cells or iPS cells). We will now determine the potential of these IPCs to normalize glucose levels in hyperglycemic mice.

Liqiud Chromatography and Capillary Electrophoresis as a Tools to Study Ligand-Receptor Interactions

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The phenomenon responsible for enantioseparation in chromatographic and electrophoretic methods is the same; it is the enantioselective interaction between the enantiomers and a chiral selector. The principal difference between these two techniques arises from different separation process mechanisms and sometimes different environments of complexation
The remarkable capacity of cyclodextrin (CD) for enantioseparation has been used to advantage in many chromatographic and electrophoretic applications
The chromatographic and electrophoretic methods which are very sensitive to structure, size, shape and dynamics of the analytes have been used not only in separation science but also in the study of molecular recognition proceses.
The enantiomeric separation of basic chiral pharmaceuticals such as pheniramine, brompheniramine, metoxyphenamine, cyclopentolate, doxylamine, and ketamine was investigated in capillary electrophoresis (CE) and liquid chromatography (HPLC) using negatively charged sulfated-β-cyclodextrin (s-β-CD) and neutral cyclodextrins (CDs). The apparent stability constants for the model compounds with cyclodextrins in both techniques were estimated.
Both methods seem to be complementary for the study of complexation phenomena. It can be seen that brompheniramine forms stronger complexes with β-CD than pheniramine and doxylamine. Complexation of pheniramine and doxylamine by β-CD is very similar. The weakest complexes β-CD forms with metoxyphenamine. For the studied compounds, TM-β-CD forms very weak complexes. The stability constant for DM-β-CD is very similar to that obtained for β-CD. From the native CDs the best chiral selectors for the studied compounds are β-CD and HP-β-CD.
For the studied compounds the best recognition between enantiomers was obtained for cyclopentolate (K_1/K_2=1.32, K_1/K_2 =1.45 and K_1/K_2 =1.26 for β-CD,for HP-β-CD and TM-β-CD, respectively)
As the CE is the more efficient method, chiral recognition is better visible in this method than in HPLC.
Conclusions: The obtained results shows that chromatographic and electrophoretic methods may be used as additional tools for studying weak interactions responsible for molecular recognition between ligand and receptor

Treatment Oucome And Management Of Acromegaly Before And After The Introduction Of Somatostatin Analogs
A long term follow-up study

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Background: Acromegaly is a rare disease resulting from GH overproduction. The available treatments for acromegaly are surgery, radiotherapy and medical treatment. Somatostatin analogs (SMS) were the first effective drugs available sinds 1980s, first as three daily injection and from 1998 as monthly depotpreparation. This drug changed the treatment strategy of acromegaly, and is now used as the first option after unsuccessful surgery, but also as primary in stad of surgery in some patients.
Methods: Long-term biochemical and clinical data are available of 164 patients diagnosed with acromegaly in the Leiden University Medical Center from 1978 onwards. We evaluated the long-term outcome of surgery, irradiation and SMS treatment in this cohort. Biochemical remission and clinical outcome, i.e. mortality, co-morbidity and quality of life parameters were evaluated. We also considered the effect of different treatment on detailed hormone secretion profile. We reviewed the effect of the introduction of SMS on the used treatment modalities in our patients, and in literature.
Results: The surgical remission rate is 66 % in short term, but there was a recurrence rate of 15 % resulting in a final remission rate by surgery only of 54% after a mean of 12 years. Radiotherapy was associated with a long-term remission rate of 75 %, however, the duration until normalization of GH excess was long and there was a high incidence of hypopituitarism. Postoperative SMS treatment was well-tolerated and resulted in a remission rate of 60 %. An iv dose of octreotide well-predicted the long-term outcome of medical therapy. Detailed growth hormone secretion was normalized following successful surgery, but not after radiotherapy and during SMS. Mortality near-normalized, but quality of life remained impaired.
Conclusions: The introduction of SMS analogs has changed the treatment algorithm of acromegaly. It is the preferred treatment in case of unsuccessful surgery and in this cohort of patients treated by surgery, radiotherapy and SMS analogs about 95% of patients achieve remission. At present equal biochemical remission rates are achieved with surgery and medical therapy only. Further study is required to assess the clinical outcome of this new treatment regimen.

Mannitol, a Key Probe Molecule in the Assessment of Small Intestinal Permeability?

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Background: The urinary recovery of orally ingested solutions containing Mannitol (mol.radius 4 Å) and Lactulose or Cr-EDTA (mol. radii 5 Å) is a widely used clinical test for small intestinal barrier function. In humans, recovery of Lactulose is ± 0.5 %, of Mannitol ± 20 %, giving a L/M recovery ratio of 0.025, which is very low compared to theorethically expected L/M diffusion ratios of 0.8 through aqueous pores Aims: To investigate the underlying mechanisms of this discrepancy by comparison of *in vivo* and *in vitro* probe permeability.
Methods: 1) Small intestinal sheets from rodents (rats, guinea pigs, rabbits) and human intestinal biopsies were mounted in Ussing chambers and mucosa-to-serosa fluxes of L/M were determined (n = 4-8). Urinary recovery of orally applied probes was measured in rodents, cats and humans (n = 2-5). 2) Absorption of Cr-EDTA, Mannitol and water was studied in *in situ* perfused jejunal loops in anaestetized cats (n = 8), using four isotonic perfusion solutions with varying contents of NaCl and glucose.
Results: L/M flux ratios *in vitro* were about 0.8 in all tested species (0.68 to 0.89). Urinary recovery L/M ratios in rodents ranged from 0.60 to 0.28. L/M ratios in cats and humans were 0.03 and 0.02, due to high mannitol recovery, resp. 29 and 22 %. In *in situ* perfused cat jejunum there was a strong positive correlation between water absorption and mannitol clearance ($r = 0.99$, $p < 0.003$), no correlation between water absorption and Cr-EDTA clearance ($r = 0.05$, $p = 0.95$). Likewise, there was a strong negative correlation between water absorption and Cr-EDTA/Mannitol ratios ($r = 0.98$, $p < 0.02$).
Conclusions: Interspecies variation in urinary recovery of mannitol is caused by differences specific for the intact small intestine *in vivo*. Hyperosmolality of villus tips *in vivo* varies, being highest in humans and cats as a result of efficient vascular countercurrent multiplication because of their villus vascular anatomical structure. Thus we hypothesize that the high mannitol recovery in both species is caused by solvent drag through pores that allow the passage of Mannitol but not of Lactulose. The positive correlation between water absorption and Mannitol clearance in cat jejunum perfused with varying solutions which differentially affect the capability of the countercurrent multiplier mechanism confirms this hypothesis.

Safe and Effective Delivery of Paclitaxel through Amphiphilic Beta-Cyclodextrin Nanoparticles

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Background: Paclitaxel is a potent anticancer drug associated with severe side effects due to the use of co-solvent Cremophor in its commercial injectable formulation. It is known that nanoparticulate drug delivery systems can provide an alternative for the effective delivery of anticancer agents due to the EPR effect facilitating the targeting of the encapsulated anticancer drug to tumor cells instead of healthy tissues. Objective of this study was to develop a nanoparticulate carrier system for paclitaxel with high encapsulation efficiency, controlled drug release property which may be more advantageous than cremophor vehicle in terms of safety and efficacy.
Methods: Nanoparticles of amphiphiphilic ß-cyclodextrins loaded with paclitaxel were prepared using nanoprecipitation technique. Particle size distribution and zeta potential of the nanoparticles were measured by QELS technique. Encapsulation efficiency of amphiphilic cyclodextrin nanoparticles were measured as well in vitro release profiles for paclitaxel with HPLC assay. Safety of the nanoparticles (nanocapsules and nanospheres) were assessed in terms of both hemolysis and cytotoxicity to L929 cells in comparison to commercial injectable formulation. Anticancer efficacy of paclitaxel loaded nanospheres and nanocapsules were evaluated by MTT assay against human breast cancer cell line MCF7 with MTT assay.
Results: Size for nanocapsules and nanospheres were found to be around 350 nm and 180 nm respectively and was found to be stable for a storage period of 12-months as seen in Figure 1. Encapsulation efficiency was increased by 2 to 3 fold by incorporating paclitaxel into cyclodextrin nanoparticles. The drug was released within 6 hours. Hemolytic order was found to be Cremophor vehicle>nanocapsules>nanospheres. Erythrocytes were imaged by SEM after treatment with paclitaxel loaded formulations. Cytotoxicity of blank nanoparticles were signficantly lower than cremophor commercial vehicle as paclitaxel demonstrated equal anticancer efficacy in nanoparticles.

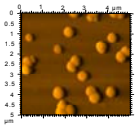

Figure 1. AFM photomicrograph of paclitaxel loaded amphiphilic cyclodextrin nanoparticles

Conclusions: Amphiphilic cyclodextrin nanoparticles can be considered as an alternative dosage form for injectable paclitaxel in terms of safety and efficacy.
Authors' disclosure statement: Author would like to acknowledge Hacettepe University Research Fund Grant 0202301005 for financial support of this study.

Two Passengers on the Cancer Road: Clomipramine and Lithium Chloride

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Background: The aim was to investigate whether a tricyclic anti-depressant clomipramine (CIM) and an anti-psychorotic lithium chloride (LiCl) potentiate the cytotoxicity of vinorelbine (VNR) at SHSH-5Y human neuroblastoma cells *in vitro* besides their conventional mode of action.
Methods: The IC_{50} values of VNR, CIM and LiCl were determined as 8 , 14.23 and 200 µM. Four sets of experiments were performed for 96 hrs both for monolayer and three dimensional (spheroid) cultures of SHSH-5Y cells. These were i) Control group, cells treated with ii) Singly applied VNR, CIM and LiCl, iii) VNR with CIM, and iv) VNR with LiCl.Their effects on monolayer cultures were determined by the evaluation of cell proliferation, the percentage of cells in S-phase by BrDU-LI, apoptosis by Annexin V-FITC/PI staining, and cAMP levels by RIA; on spheroid cultures by evaluation of the percentage of cells in S phase, spheroid size, and the ultrastructure by TEM.
Results: In comparison to the control group, single and combination drug medications significantly reduced the proliferation index (PI) for 96 hrs. The most potent reduction of PI was observed at VNR with CIM and LiCl for all time intervals. VNR with CIM and LiCl seemed to be ineffective to reduce BrDU-LI of both monolayer cell and spheroid cultures, spheroids size, cAMP levels. VNR with LiCl increased apoptosis potently at 24 hrs, however VNR with CIM increased apoptosis at 96 hrs. In ultrastructural evaluation of spheroids, increased presumably autophagic vacuoles, mitochondria damage and debris of lytic cells in extracellular area were observed at VNR with CIM. Striking data were catched at VNR with LiCl applied spheroids. The VNR applied spheroids revealed intact nuclear and cellular membranes. LiCl led to nuclear membrane breakdown at nearly all cells of spheroids. When VRL was used with LiCl, in addition to nuclear membrane breakdown, the cellular membranes inside spheroids disappeared and the cellular membranes were appeared as an unique membrane constructions only in spheroid surface. As a result of this, one spheroid resembled one giant cell.
Conclusions: 1) Both CIM and LiCl seemed to potentiate VNR-induced cytotoxicity with few exceptions. **2)** Interestingly, VNR with LiCl led to selective nuclear and cellular membranes destruction. This effect could be due to the premature activation of cyclin dependent kinase 2.

Analysis of drug-receptor interaction at equilibrium

BINDSLEV N

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Aim: We want to analyze our dose-response data at equilibrium by models. We may have an idea about a possible physical interpretation of obtained response curves and use either mathematical or mechanistic descriptions.
Background: From 1901 to 1910, receptors as separate entities were recognized by Elliott (1904) and Langley (1905) in Cambridge and Ehrlich (Frankfurt 1907). Deviation from a simple Langmuirian hyperbole (1918) was documented by C. Bohr (Copenhagen 1904) for oxygen-hemoglobin binding.
Mono-ligand systems: For mono-ligand systems with dose-responses deviating from a simple hyperbole, Hill=s equation can give you a quantitative measure of co-operativity; but without assessing possible interactions between binding sites. Therefore, co-operative values from a Hill scheme most likely has no physical correlate.
For a physical description of systems deviating from simple hyperbolism, I suggest the homotropic two-state model, HOTSM (2004). This model can handle both positive and negative co-operativity as well as bell-shaped and reverse bell-shaped relationships.
Two-ligand systems: Models for two ligands may again be based on either a Anon-interaction@ scheme such as the non-competitive inhibition scheme without two-states of the un-liganded receptor or on an extended Monod-Wyman-Changeux scheme (Monod et al 1965; Rubin & Changeux 1966) with two-states. Both these approaches assume no interaction between binding sites, i.e., upon binding of a ligand there is no change in the binding constant for a second drug.
Instead, for combinatorial drug effects with assumed site interaction, analysis of data may be performed by the ternary complex model, TCM, a one-state scheme for two ligands with site interaction (Ehlert 1988), or alternatively by the allosteric two-state model, ATSM, with site interaction (Hall 2000).
ATSM implementation: As one of the first, Jäger et al have implemented the ATSM (2007). Thus, physical effects are quantified for naphmethonium on acetylcholine binding and on pilocarpine activation of an M2 receptor subtype. Lately, Ehlert-Griffin (2008) have extended the ATSM with an additional ligand. A tri-ligand two-state model.
Conclusion: Depending on the synagic system to be analyzed, i.e., the actual dose-response relationships at equilibrium, we may choose between Hill, Hall or other models. A critical choice is crucial for our extraction and use of relevant mechanistic parameters.

The Pharmacokinetics (PK) of Voriconazole in Children

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Background: Invasive fungal infections are a major cause of morbidity and mortality in immuncomprimised patients. The most common fungi responsible for severe infections are *Aspergillus*. Voriconazole is a broad spectrum second generation triazole antifungal agent. It is indicated for the treatment of invasive aspergillosis. Pediatric dosage finding and safety evaluations have not been completed. Aims: 1)The aim of this study was to review the literature about studies done on the PK of voriconazole in children. 2) The clinical results of our 5 cases with *Aspergillus* infection (4 pulmonary, 1 bloodstream) with voriconazole treatment were shown.
Methods: Literature was searched with the key words "voriconazole, pharmacokinetic, children, invasive fungal infection" and the studies were evaluated.
Results: Children require higher doses of voriconazole than adults to attain similar serum concentrations over time because the drug exhibits non-linear pharmacokinetics in adults, but exhibits linearity in children. A significant relationship between disease progression and drug concentration was described in adults (Antimicrob Agents Chemother 2006;50.1570). Based upon studies in children, it appears that a pediatric dosage of 11 mg/kg administered every 12 h is approximately bioequivalent to an adult dosage of 4 mg/kg given 12 h. Plasma samples for voriconazole HPLC assay from 14 subjects revealed that in children receiving dosages of ≥4 mg/kg iv bid was lower than that of adult volunteers receiving 4 and 5 mg/kg bid (Walsh TJ, et al). In another study done in 5 children with ages ranged from 2 to 10 years old voriconazole was administered at dosages varied from 3.4 mg/kg every 12 h to 8.1 mg/kg every 8 h and plasma voriconazole concentrations were found to be unpredictable for these paediatric patients (Arch Dis Child 2008;93:578). Therefore, using voriconazole at recommended doses for adults may lead to clinical failures in children. Also, recent observations suggest that hepatic toxicity and visual disturbance might be dose related. Five of our cases treated successfully without any major side effect with voriconozole (14 mg/kg/d) will be presented.
Conclusions: 1) Children might require higher doses of voriconazole than adults to attain similar serum concentrations over time because of its linear PKs in children. Studies about the optimum dosage of voriconoazole in children should be conducted in a large number of children.

Modification of the Infarct Size Limiting Effects of Statins by Antiplatelet Drugs

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Background: Therapy for acute coronary syndromes includes statins and anti-platelet agents. Statins limit infarct size (IS) in animal models by activation of phosphoinositide-3-kinase (PI3K) with subsequent activation of ecto-5-nucleotidase (that generates adenosine) and Akt/endothelial nitric oxide synthase (eNOS) with downstream activation of inducible NOS (iNOS) and COX2. Inhibition of the PI3K, adenosine receptors, eNOS, iNOS and COX2 abrogates the IS-limiting effects of statins.
Methods: Rats received 3-day oral atorvastatin (ATV) or vehicle with or without dipyridamole (DIP, 6mg/kg/d), cilostazol (CIL, 20mg/kg/d), or aspirin (ASA, 5, 10 or 20mg/kg at reperfusion). Rats underwent 30-minute coronary artery occlusion and 4-hour reperfusion.
Results: ATV (10mg/kg/d) limited IS. Intravenous ASA before reperfusion attenuated this effect. DIP alone and ATV (2mg/kg/d) alone had no effect on IS; however, IS was significantly reduced in the ATV+DIP combination. Myocardial adenosine levels were higher in the ATV+DIP group than in the ATV alone, DIP alone and the control group. The protective effect was abolished with theophylline, indicating that it is mediated by adenosine receptor activation. CIL alone, and especially when combined with ATV (2 mg/kg/d) limited IS. CIL increased myocardial levels of adenosine and Akt and eNOS phosphorylation. In addition, by increasing tissue cAMP levels, CIL activated protein kinase- A that phosphorylates eNOS. CIL inhibited PTEN, thus leading to augmentation of Akt and subsequently eNOS phosphorylation.
Conclusions: Aspirin blocks the IS-limiting effects of statins, whereas both dipyridamole and cilostazol have synergistic effects with statins. It might be that the anti-platelet regimens should be modified for patients receiving statins.

Structure and Function of the Ubiquitous TRPC channels: Targets in Need of Magical Bullets.

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The canonical transient receptor potential (TRPC) channels were discovered in our laboratory in 1995-96. They are homolgues of the Drosophila light-activated channels Trp and Trp-like and were cloned to test the hypothesis that in mammalian cells they might be at the root of not only Gq-activated non-selective cation currents but also store-operated Ca^{2+} entry (SOCE). In initially 6, now 7, TRPCs have distant homology to voltage-gated cation channels and span the membrane 6 times. Between their discovery and now, the participation of TRPCs as members of SOCE channels has been controversial and indirect. An alternative hypothesis emerged after the discovery in 2005 of STIM, a 1-pass Ca^{2+}-sensing membrane protein located in the ER that organizes plasma membrane SOCE channels, and in 2006 of Orai, a 4-pass plasma membrane protein whose loss is responsible for a familiar form of severe combined immunodefficiency and whose expression together with STIM engenders in TRPC expressing cells very large SOCE activity. This alternative hypothesis postulates Orai as the SOCE channel activated by STIM and does not include a role for TRPCs. However, as we published in Jan 2007, Orai and TRPCs interact functionally, seen as a TRPC-dependent enhancement of SOCE upon expression of low levels of Orai . We proposed that instead of forming channels, Orai appear to be regulatory proteins that confer Ca^{2+}-selective SOCE channel properties to the otherwise non-selective cation channels formed by TRPCs without Orai. In support, SOCE channels were shown in other laboratories to be dynamically assembled in lipid rafts from which TRPCs and Orai can be co-immunoprecipitated. A survey of the literature shows that physiologic roles for TRPCs have been proven in the central nervous system, in the cardiovascular system, in the kidney, in epithelia, endothelia and blood-borne cells, and in various muscle types. TRPCs act in these tissues either by depolarizing their membrane or through the Ca^{2+} they allow to enter upon association with Orai. The latter includes cell proliferation. TRPCs play roles in human diseases, including cancer. TRPCs and Orai are important targets waiting for the development of Magic Bullets that will help in better understanding their role and in ameliorating diseases involving altered TRPC and/or Orai funcitons.

Starvation and Oxidative Stress as an Inductor of Ciprofloxacin Resistance

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Background: Mutation rate of bacteria is often affected by environmental conditions. Various stress such starvation, oxidative or radiating stress can result in increased frequency of mutations leading to antibiotic resistance. The aim of this work was to determine the mutation frequency leading to ciprofloxacin resistance induced by depleted media and hydrogen peroxide and to find molecular determinants of ciprofloxacin resistance in selected mutants.
Methods: Oxidative stress was evoked by 3h cultivation of *S. typhimurium* with hydrogen peroxide in 3 concentrations (0.4, 0.8, 2, 4 mM). Starvation was studied after incubation (3h) of bacteria in control Luria-Bertani medium (LB) and in nutritionally depleted media 10%LB and Nutrient broth No. 1. Resistant strains were counted on agar plates supplemented with ciprofloxacin (0.06 mg/ml) after 72 h of incubation at 37°C. The frequency of resistant mutants (resistance index RI) represents mean number of resistant cells divided by the total number of viable cells per culture. Data represent the mean of three independent experiments; each experiment was made in five parallels and statistically evaluated by Student's t-test. Mutations in *gyrA* were determined using AS-PCR-RFLP method. Levels of outer membrane porin F were detected with SDS-PAGE.
Results: Short-term cultivation in 10% LB caused 430 fold increase of RI while in Nutrient broth No.1 it was only 57 fold rise. In ciprofloxacin-resistant strains generated by long-term starvation were detected decreased levels of OmpF protein. With rising dose of H_2O_2 was RI increasing up to 33-fold of spontaneous mutation frequency to ciprofloxacin resistance. In nutritionally depleted medium with H_2O_2 has mutation frequency increased more than 103-time. 80% of resistant strains had mutation in *gyrA*. 37% of them had mutation in codon Ser-83 and 63% in codon Asp-87.
Conclusions: 1) Starvation increases mutagenesis leading to ciprofloxacin resistance. 2) Short-term treatment of *S.typhimurium* in nutritionally depleted media caused higher increase of ciprofloxacin RI than long-term incubation.3) Long-term starvation is leading to decreased levels of OmpF. 4) Oxidative stress induced by H_2O_2 in conjunction with lack of nutrients in environment increases mutation frequency to ciprofloxacin resistance. 5) Majority of ciprofloxacin-resistant strains generated by H_2O_2 has mutation in *gyrA* gene.

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Identification of the anti-inflammatory targets of interactive constituents of *Hypericum perforatum* (Hp).

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Background: Hp is used as a botanical therapy for infective disorders. The level of constituents in Hp extracts were lower than the concentration of pure constituents needed to reduce lipopolysaccharide (LPS)-induced prostaglandin E_2 (PGE_2) production in RAW 264.7 macrophages; suggesting an interaction of compounds for the activity. The goal of this study was to identify key constituents and investigate the gene targets for the anti-inflammatory effect.
Methods: A flavonoid-rich bioactivity guided fractionation was used with screening in the LPS-induced RAW 264.7 macrophage system to identify active fractions from each round and liquid chromatography-mass spectrometry (LC-MS) identified constituents present. CellTiter96™ Aqueous solution revealed no significant cytotoxicity with fractions or constituents at the doses studied. Microarray analysis was performed with Hp fraction, the 4 constituents combined at levels detected in the Hp fraction, and solvent control with/without LPS.
Results: A third round fraction (3A) (10 µg/ml) significantly reduced PGE_2. Combining four constituents at concentrations detected in the LC-MS analysis (0.17 µM chlorogenic acid, 0.08 µM amentoflavone, 0.08 µM quercetin, and 0.03 µM pseudohypericin (PHCN) explained the anti-inflammatory activity of the fraction in light-activated conditions. The amount of each pure constituent needed to observe a significant reduction in PGE_2 was > 50 times more than was found in fraction 3A. Of the 4 interacting compounds, only PHCN was required. With LPS, the 4 component system affected 162 genes and the fraction affected 780 genes; 40 genes were differentially expressed under both treatments. Important pathways for both treatments were the Janus kinase-signal transducer and activator of transcription and eicosanoid metabolism pathways.
Conclusions: 1) Interactions of constituents explained the anti-inflammatory activity of fraction 3A. 2) PHCN was required for the anti-inflammatory activity in combination with one or more other constituents. 3) The gene targets identified for the 4 component system were consistent with the reduction of LPS induced PGE2. This work supports the role of interactions of compounds in Hp targeting genes that are important in inflammation. Supported by grants ES012020 from NIEHS/ODS and 9P50AT004155-06 from NCCAM/ODS, NIH.

LC-MS for Label-Free Biomarker Discovery

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Background: The discovery and validation of biomarkers is an important goal in many areas of biomedical research and drug development. Changes in individual proteins or peptides as well as in protein and peptide profiles in bodily fluids or tissue biopsies have been implicated in diagnosis and prognosis of various diseased states. Recent developments in analytical chemistry allow to obtain complex, quantitative data from hundreds to thousands of compounds (proteins, peptides, metabolites) that require novel data processing and statistical approaches to arrive at biomarker candidates.
Methods: Serum was prepared for proteomics analysis by Liquid-Chromatography Mass Spectrometry (LC-MS) [1]. The generated data were processed using in-house developed algorithms [2,3] and analyzed by multi-variate statistics in combination with variable selection algorithms.
Results: LC-MS analysis of sera from cervical cancer patients were analyzed prior to and approximately 6 months after therapy. While differences in protein profiles were minor for early-stage disease differences became significant for more advanced stage tumors. Follow up of patients over multiple years showed that certain biomarker candidates changed in agreement with the recurrence of disease. Some of these candidates were related to the glycosylation of serum proteins.
Conclusions: 1) Biomarker candidates have been discovered that correlate with the response to therapy for cervical cancer. 2) Dedicated data processing and statistical analysis reduced the number of variables to a number that is in-line with the number of analyzed samples.
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Reactive Oxygen Species Have a Causal role in the Development of Insulin Resistance in Diabetic, Hypercortisolemic and Chronic Inflammatory States: Amelioration with the Antioxidants Alpha-lipoic acid

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Background: Insulin resistance, featured by an inexorable decline in skeletal muscle glucose utilization and/or an excessive hepatic glucose production, represents a major pathogenic importance in a cluster of clinical disorders including diabetes mellitus, hypercortisolemia, inflammation, and coronary artery disease. A novel concept suggests that excessive generation of reactive oxygen species (ROS) contribute to the development of insulin resistance in most if not all the aforementioned disease states.
Methods: Euglycemic-hyperinsulinemic clamp studies with an insulin infusion index of 5 mU/kg bw/min were used to measure endogenous glucose production (EGP), glucose infusion rate (GIR), glucose disposal rate (GDR) and skeletal muscle glucose utilization index (GUI). Moreover, the status of oxidative stress as reflected by urinary levels of isoprostane and tissue contents of protein-bound carbonyls and thiobarbituric acid reactive substrates (TBARS) were also assessed as a function of diabetes, hypercortisolemia and chronic low grade inflammation.
Results: Post-absorptive basal EGP and circulating levels of insulin, glucose and free fatty acid were elevated in GK and to a lesser extent in Dex and TNF alpha-treated rats,compared to their corresponding control values. In contrast, steady state GIR and GDR of the hyperglycemic/hyperinsulinemic animals were reduced,concomitantly with impaired insulin‘s ability to suppress EGP. The suppression of skeletal muscle glucose utilization in these animals was associated with a decrease in insulin‘s ability to promote the phosphorylation of tyrosine residues of insulin receptor substrate-1. Similarly, the translocation of glucose transporter-4 from intracellular compartment to plasma membrane in response to insulin was also reduced in these animals. Oxidative stress-based markers (e.g. urinary isoprostane, carbonyl-bound proteins, TBARS) were elevated in response to diabetes, hypercotisolemia and chronic low grade inflammation. Nullification of the heightened state of oxidative stress in the aforementioned animal models with antioxidants such as alpha lipoic acid ameliorated hepatic and skeletal muscle insulin resistance.
Conclusions: Collectively, the above data suggest that excessive generation of ROS in connection with their detrimental effects on lipid and protein molecules have a causal role in multiple forms of insulin resistance.

Synthesis of Unnatural Ceramide Analogs and Their Antiproliferative Properties Against a Panel of Cancer Cells

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Abstract: 2*S*,3*R*-Ceramide **1** occupies the “hub” of sphingolipid metabolism and serves as a coordinator of eukaryotic stress responses and other biological activities such as cell growth and differentiation. Ceramide **1** plays a key role in programmed cell death (apoptosis). However, since **1** is a naturally occurring lipid, it is recognized by endogenous enzymes and can be converted into anti-apoptotic lipids via phosphorylation and glycosylation. We sought to prepare unnatural ceramide analogs that may be longer lived in cells because they are unrecognized by enzymes. We found that some of the unnatural ceramide analogs have greater antiproliferative activity than **1** against human breast cancer cell lines in vitro. One of the unnatural ceramide analogs we synthesized has an exocyclic double bond in the sphingoid base (compound **2**), whereas another has a disulfide linkage in the *N*-acyl chain (compound **3**). Their antiproliferative activities against three human breast cancer cell lines (BT549, MDA-MB-231, MCF-7), a lung cancer cell line (A549), a prostate cancer cell line (DU145), and a cervical cell line (HeLa) were analyzed in vitro and compared with the activity of (2*S*,3*R*)-*N*-octanoylceramide (**1**). The sulfur-containing-ceramide analog **3** and the exo-ceramide analog **2** exhibited a higher antiproliferative activity than natural ceramide **1**. Caspases in cells treated with compound **3** were activated, indicating that the cells underwent apoptosis. A ceramide analog containing a tetrahydrofuranyl ring (compound **4**) and a phytoceramide analog (compound **5**) exhibited a much higher antiproliferative activity than natural N-palmitoyl-D-*erythro*-ceramide against T47D, MCF7, and MDA-MB-468 cells. The syntheses, antiproliferative activity, and mechanistic studies of the apoptotic properties of these ceramide analogs will be presented.

Peltophorum africanum Sond (Fabaceae) has a role in disease control

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Background. About 80% of people in the developing world, particularly those from rural communities where modern drugs are unaffordable, inaccessible or, unavailable, depend on phytomedicine for primary healthcare. However, most medical and veterinary professionals distrust herbal medicines due to lack of scientific evidence of efficacy and safety. Hence, there is need for their validation, before herbal medicines gain wider acceptance and use. Traditional healers, pastoralists and rural farmers use extracts of *Peltophorum africanum* (a medicinal plant widely spread in southern Africa and other tropical regions), to treat diarrhea, dysentery, pain, infertility, HIV-AIDS and to promote well-being and resistance to disease. The extracts of the plant inhibit HIV-type 1 reverse transcriptase and protease.
Methodology. Dried leaves bark and root from mature *P. africanum* trees were extracted with acetone. Chromatograms were made on silica gel plates. Minimum inhibitory concentrations (MIC) were determined for five bacteria (Gram positive and Gram negative), and five fungal pathogens. Qualitative screening for antioxidants was done by spraying chromatograms with 0.2% 2, 2-diphenyl-1-picryl hydrazyl (DPPH) , and quantification done in comparison with L-ascorbic acid and Trolox (6-hydroxy-2, 5, 7, 8-tetranethylchromane-2-carboxylic acid). Anthelmintic activity was evaluated by effects of extracts on the egg hatching and larval development of parasitic nematodes *Haemonchus contortus* and *Trichostrongylus colubriformis*.
Results. The extracts showed substantial activity against both Gram-positive and Gram-negative bacteria, with Minimum Inhibitory Concentration (MIC) values of 0.08 mg ml^{-1} for *Staphylococcus aureus* and 0.16 mg ml^{-1} for *Pseudomonas aeruginosa*. The extracts showed higher antifungal activity than amphoterin B. The acetone extracts of the bark, and root of *P. africanum* showed higher antioxidant activity than L-ascorbic acid (Vitamin-C) and Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid), a synthetic vitamin-E analogue, and much higher than *Ginkgo biloba* extract (EGb 761). The respective EC_{50} for the *P. africanum* root and bark extracts, L-ascorbic acid, and EGb761 were 3.82µg/ml, 4.37µg/ml, 5.04µg/ml, and 40.72 µg/mL. The standardised extract of *Ginkgo biloba* (EGb 761) is widely employed for its significant benefit in neurological disorders. The extracts inhibited egg hatchability and larval development (from L_1 to infective stage L_3) of both *Haemoncus contortus* and *Trichostrongylus colubriformis* (both parasitic nematodes of ruminants) at concentrations of 0.1-1.0 mg ml^{-1}. The plant extracts, at the concentration of 5-25 mg ml^{-1} completely lysed larval forms (L_1) and eggs of the nematodes.
Conclusion. *P. africanum* extracts have therefore, potential for treatment of inf7ction-related diseases by either directly inhibiting bacterial growth or by stimulating the immune system of the host. The traditional use of *P. africanum* concoctions against diarrhea, dysentery and unthriftness, may be also due to anthelmintic activity as these signs are consistent with parasitic gastroenteritis. Gastrointestinal nematodes exasperate diarrhea in HIV-AIDS patients, as well as disease-related production losses arising from stock mortality, severe weight loss and poor production in ruminants. Antioxidants are also important in boosting the immunity, critical in the management of helminthosis. There is ample scientific and empirical evidence supporting the use of plant-derived antioxidants in the control of neurological diseases, as antioxidants have neuro-protective (preventing apoptosis), as well as neuro-regenerative roles. Due to the high antioxidant activity of its extracts, *P. africanum* has prospects in the management or control of neurodegenerative diseases. Thus there is great potential of *P. africanum* extracts in disease control.

Brain cholesterol? Long secret life behind a barrier

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The blood-brain barrier is almost completely impermeable for cholesterol in both directions. All cholesterol present in the brain is thus a product a local synthesis. Since there is a low but significant synthesis of cholesterol in the adult mammalian brain we hypothesized that there may be a compensatory flux of a cholesterol metabolite from the brain across the blood-brain barrier. About 14 years ago we identified this metabolite as 24S-hydroxycholesterol (24OHC) and showed that in contrast to cholesterol itself, this oxysterol can cross the blood-brain barrier. By measuring the concentration difference between the internal jugular vein and an artery in human volunteers, we demonstrated the production of 24OHC by the brain to be about 6 mg/24h. Since the uptake of 24OHC by the liver was fund to be about the same, it is evident that almost all 24OHC in the circulation originates from the brain.
In spite of the fact that cholesterol does not pass the blood-brain barrier, hypercholesterolemia is a risk factor for Alzheimer Disease (AD). We hypothesized that there may be a metabolite of cholesterol fluxing in the opposite direction from the circulation into the brain. This metabolite was identified as 27-hydroxycholesterol (27OHC), also using the catheterization approach. The uptake of this oxysterol by the human brain was found to be about 5 mg/24h. Since there is a good correlation between levels of cholesterol and 27OHC in the circulation, it seems likely that the uptake of 27OHC by the brain is related to the levels of cholesterol in the circulation.
In spite of the relatively high influx, levels of 27OHC in the brain are very low, indicating an efficient metabolism. The major metabolite was identified as 7a-hydroxy-3-oxo-4-cholestenoic acid. This acid very efficiently passed a model for the blood-brain barrier and we found a net flux of it from the human brain into the circulation. The conversion of 27OHC into the steroid acid can be regarded as a regulated detoxification. 27OHC is an efficient suppressor of cholesterol synthesis and we have shown that the compound is able to increase amyloid formation in neuroblastoma cells.

Different pathogenetic aspects of the oxysterol crosstalk over the blood-brain barrier will be discussed in the lecture and it is suggested that the flux of 27OHC from the circulation into the brain is the missing link between hypercholesterolemia and AD.

Therapeutic Differentiation of Insulinoma Cell Lines Treated with Streptozotocin

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Background: Streptozotocin (STZ) is a member of a group of alkylating antineoplastic drugs, and is clinically active against insulinomas. STZ toxicity depends on glucose transporter protein-2 (GLUT-2) expression and generation of free radicals. As with many other chemotherapeutic drugs, repeated treatment with STZ, may induce a selection of resistant cell populations. Differentiation therapy has been proposed as a promising approach to selectively engage the process of tumor cell differentiation during chemotherapy. According to this approach, cytotoxic agents can induce drug resistance, but in certain conditions, can also lead to recovery of normal cell homeostasis. Aim: To estimate toxin resistance and differentiation of insulinoma cell survival following exposure to STZ.
Methods: A parental highly differentiated mouse insulinoma cell line (BTC-tet) and low differentiated rat insulinoma cell line (RINm) were repeatedly exposed to STZ. The cell populations (RIN-S and BTC-S) surviving such treatment were examined as to their resistance to STZ, hydrogen peroxide, nitric oxide and cytokines. Western blot analysis was applied to estimate GLUT-2 and bcl-2 expression in parental and STZ selected cells. Insulin content and secretion, cell proliferation, morphological and chromosomal characteristics were studied.
Results: Repeated STZ treatment of parental insulinoma cell lines resulted in the selection of cell sub-populations with multiple resistance to different toxins. The enhanced toxin tolerance may be explained by a low level of GLUT-2 and high expression of bcl-2 in selected cells. In addition, STZ selected cells displayed a lower rate of cell proliferation when compared to untreated cells. Moreover, BTC-S and RIN-S cells showed 2-5 times higher levels of intracellular insulin content and improved insulin response to glucose than their parental cells. STZ-based selection changed cell morphology and increased frequency of polyploidy in RIN-S (2.2%) compared to RINm cells (0.7%).
Conclusions: Repeated STZ treatment of insulinoma cell lines results in the selection of cell subpopulations possessing multiple toxin resistance, a low rate of proliferation and enhanced functional activity. Further characterization of STZ selected beta cells could provide useful lessons for optimization of differentiation therapy of cancer.

Development of a new 3D-Human Airway Epithelium/ Whole-blood Co-culture Model Combined with Multi-Analyte Profile (MAP) Analyses for Assessing Drug Effects

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Background: The dialogue between cells of the immune system and cells of various tissues controls immune reactions and is in part mediated by a variety of cytokines, chemokines etc. This network may be strongly influenced by the application of drugs. Aim of our investigations was to develop an innovative organo-typical human airway epithelial co-culture model for the analysis of immunopharmacological activities of drugs.
Methods: A differentiated airway epithelium, MucilAir, was combined with whole-blood cultures in a two-chamber system to study the effects of betamethasone applied onto the epithelium sitting on activated immune cells from a healthy donor mimicking an inflamed tissue environment. 92 mediators and other parameters were tested in the supernatants of the cell cultures by multiplexed bead assays (RBM MAP analysis).
Results: Betamethasone exhibited its typical, strong pharmacological effect profile on both, the immune and the epithelial cells: It dose-dependently inhibited a variety of pro-inflammatory mediators, being either T helper cell type 1- (Th1), Th2-, or macrophage-associated, such as interferon (IFN)-γ interleukin (IL)-5 and tumor necrosis factor (TNF)-α, respectively. In contrast, IL-10 as a mediator of regulatory T cells was up-regulated after 24h of co-culture. Furthermore, epithelial cells were cultured for another 6 days showing a dose-dependent effect on e.g. the monocyte chemotactic protein-1 (MCP-1).
Conclusions: From the data we will present in this poster, it is evident that the highly complex, organo-typical co-culture model provides an excellent, *in vivo*-like tool to study *in vitro* not only the pharmacokinetics and pharmacodynamics of inhaled drugs, but also the harmful effects of toxicants that get access to the human lung.

Drug Potential of Nigerian MedicinE Related Plants: A SURVEY

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Medicinal plants are plants used whole or parts to prevent and cure health problems, promote and rehabilitate nature to the living population at primary, secondary and tertiary health care deliveries. In Nigeria, the use of plants at various levels of health care delivery has been in practice for many centuries past; as old as the history of human beings. These plants belong to several families including Leguminosae, Malvaceae, Mimosaceae, Euphorbiaceae, Compositae, Acanthaceae, Cannaraceae, Passifloraceae, Rutaceae, Zingiberaceae, Bombacaceae, Olacaceae, Apocynaceae, Guttiferae, Liliaceae, Sapindaceae and Combretaceae. Specific examples of the phyto-organisms are Pericopsis, Phaulopsis, Myrobalan, Pleiocarpa, Picralima, Fig, Bhadram, Akerbia, Millefoil, Bear?s breach, Copper leaf, Acalypha, Acacia, African mallow and Baobab. sThese plants are used for different medicinal purposes such as stimulant and carminative, avert fever, coughs, asthma, flatulence, helminthic and microbial problems, cancer, hypertension, leprosy, vitamin deficiency, lack of homeostasis, pox and ulcer.

Purposeful Drug-Excipients Physico-Chemical Interactions – What does that mean for Optimization of Drug Delivery and Safety?

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Background: Drug-excipients interactions attract research interest as a biopharmaceutical tool to influence the onset, intensity and the duration of drug performance *In vivo*. Aim: to improve the dissolution characteristics and optimize *In vitro* drug release profile of some non-steroidal anti-inflammatory drugs - indomethacin (IND), ibuprofen (IBP), naproxen (NAP) by *In vitro* interactions with hydrophilic polymers - polyvinyl(pyrrolidone) (PVP), polyethyleneglycol (PEG), hydroxyethyl cellulose (HEC), sodium alginate, dextran and silica-PVP- or silica-methylmetacrylate nanohybrids.
Methods: Different pharmaceutical techniques – solid dispersions-, adsorbates formation, sol-gel reactions and appropriate modern physico-chemical (FT-IR, X-ray, DSC, solid state C13 and Si29 NMR, AFM, TEM) - and in silico methods for characterization have been applied.
Results: Changes in physico-chemical properties of drugs have been achieved - polymorphic transitions (IND), inhibition of crystallization and amorphization (IND, IBP), complex formation (IND,IBP), particle size reduction. All changes are related to significant increase of In vitro drug dissolution. Special emphasis has been put upon the elucidation of the character of occurring interactions on molecular level. The modern sol-gel technique has been applied to develop Silica-PVP-IBP model nanohybrids of prolonged, pH-independent IBP release.The main advantage of this reaction is that it takes place at mild experimental conditions and enables drug immobilization into the inorganic-organic network.
Conclusions: Physico-chemical transformations of some non-steroidal anti-inflammatory drugs by means of purposeful interactions with polymers and nanohybrids have been proven. Significant improvement of *In vitro* drug dissolution and modified drug release profiles were registered. The results give reasons to assume an increase of *In vivo* drug activity and safety.

Escitalopram-the first ASRI. A Magic Bullet in the treatment of depression and anxiety.

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Selective Serotonin Uptake Inhibitors (SSRI's) are drugs (bullets) that are targeting the serotonin transporter in order to block the reuptake of the neurotransmitter serotonin. Escitalopram has, in contrast to other SSRI drugs been shown to interact with the serotonin transporter at two different sites: the primary binding site shared with other SSRI's and a separate allosteric binding site. A consequence of this unique allosteric interaction is a prolonged dissociation half-life from the primary binding site resulting in very efficient inhibition of the serotonin uptake. Escitalopram is therefore due to this self-enhancing effect a Magic Bullet which has been termed Allosteric Serotonin Uptake Inhibitor (ASRI).

Escitalopram is the active enantiomer of the racemic drug citalopram. While the R-enantiomer originally was believed to be inactive, recent investigations have surprisingly show that the R-enantiomer inhibit the effect of the S-enantiomer. Pharmacological and clinical studies have shown a clear tendency for faster onset of antidepressant action and more than two-fold higher potency of escitalopram compared with citalopram.

The presentation will focus on comparing the profile of escitalopram/ASRI with SSRI's and discuss the mechanism behind and the consequences of the inhibition of S-citalopram by its R-enantiomer. A molecular model of the serotonin transporter will be presented as well as possible mechanisms involved in the allosteric interaction.

The influence of cytarabine and myristic acid on aspirin binding with serum albumin. Spectroscopic study

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Background: In the body drugs are transported mostly via circulatory system as the complexes with albumin. This is an important part of the drug metabolism since the bound fraction of a drug has no pharmacological effect. The simultaneous binding of other exo- and endogenous ligands (such as drugs or fatty acids) can alter protein affinity towards drug and change the concentration of its free fraction. In our study the influence of cytarabine (araC) and myristic acid (MYR) on aspirin (ASA) binding with defatted bovine serum albumin (dBSA) was investigated.
Methods: NMR spectra were recorded on Bruker Avance 400 spectrometer using 5 mm tubes. For water signal suppression the presaturation method was used. All solutions were prepared in D2O. The chemical shifts of proton resonances were calculated in relation to DSS signal (0.05 ppm).
Fluorescence emission spectra were recorded on Kontron SFM 25 spectrofluorimeter at excitation wavelength of 280 nm. Binding constants Ka were calculated with the use of Scatchard method. All measurements were obtained at 310K.
Results: The association constants calculated for the first (subdomain IIA) and second (subdomain IIIA) class of ASA binding site is $84{,}2x10^3M^{-1}$ and $1{,}81x10^3M^{-1}$, respectively.
^{1}HNMR spectra show that dBSA and araC induce changes in chemical shifts of ASA proton resonances. The effect is more evident for the aromatic ring of ASA participating in the formation of the complex than for its methyl group. Changing of chemical shifts of ASA proton resonances are not enough significant to observe differences in drug binding in the presence of MYR.
Spectrofluorimetric analysis indicate that above [MYR]/[dBSA] molar ratio 4:1 K_aI and K_aII of ASA-dBSA complex decrease approximately by 50 and 35%.
Conclusions: 1. ASA is displaced by araC in both high and low affinity binding sites. 2. Binding of MYR with albumin influences formation of ASA complexes with albumin. This effect depends on MYR:dBSA molar ratio. 3. In case of multidrug therapy or hyperlipidemia the monitoring therapy may be necessary.

Current systemic and local therapies of intra-abdominal sepsis

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Abstract
Intra-abdominal sepsis (IAS) or infection is defined as inflammation of the peritoneum due to pathogenic microorganisms and their products. IAS is not a local disease, affecting the entire body, its organs and systems, producing systemic inflammatory response syndrome (SIRS). A favorable outcome for IAS depends on the ability of host defenses to overcome pathogenic microorganisms. The current treatment of IAS consists of: 1. surgical control of the source of infection, 2. reduction of intra-abdominal bacterial inoculum and 3. supportive measures. The pharmacological and mechanical means for combating intra-abdominal bacteria and the combination of the above measures will be reviewed. The systemic and local strategies to treat primary, secondary and tertiary forms of IAS will be discussed.

A generic, multidimensional SPE-platform for undisturbed LC-MS/MS analysis of basic drugs in native biofluids

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Background: In bioanalytical LC-MS electrospray ionization is very susceptible to so called matrix effects that are caused by coeluting low (LMW) and high (HMW) molecular weight sample components. Due to the natural variation of endogenous compound concentrations in biofluids such as urine and blood plasma within an individual, between individuals and between species, matrix effects are hardly predictable. In order to minimize matrix effects we treated the native biofluids by on-line multidimensional Solid Phase Extraction (MD-SPE) prior to on-line LC-MS/MS.
Methods: The MD-SPE platform (High SPEed, CTC Analytics) relies on the combination of two SPE columns. The first SPE column (25 x 2 mm ID) is packed with Restricted Access Material (RAM; LiChrospher ADS RP 4, dp 25 µm, Merck KGaA) which allows size-exclusion (SEC: 1st dimension) and reversed phase chromatography (RPC: 2nd dimension). The second SPE column (20 x 2.1 mm ID) contains a mixed-mode phase (MMP; Oasis WCX, dp 30 µm, Waters) which allows weak cation exchange (IEX: 3rd dimension) and hydrophobic interaction chromatography (HIC: 4th dimension). Analytical separation of the model analytes (antidepressants) was achieved on a Zorbax RX C18 column (75 x 4.6 mm ID, dp 3.5 µm, Agilent). A Quattro Micro MS (Waters) was used in ESI(+) MRM mode. Matrix effects were monitored in post-column infusion experiments.
Results: The RAM-SPE column repeatedly and very efficiently depleted plasma samples (20 µL) with regard to HMW matrix components such as proteins and quantitatively extracted the target analytes (pka value higher than 6.5) in less than 60 sec. After transfer onto the MMP-SPE column residual LMW matrix components (< 15 KDa) were eluted to waste within 60 sec. This extensive and highly selective sample clean-up resulted in almost no detectable matrix effects. In addition, MS-scans (m/z range from 100 to 1000) of blank plasma and urine from different individuals and species revealed no masses, i.e. no residual matrix components [1].
Conclusion: A combination of orthogonal chromatographic separation modes, i.e. SEC-RPC-IEX-HIC, for on-line SPE of native biofluids such as plasma and urine completely eliminates matrix effects in LC-ESI-MS/MS detection of basic drugs.

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Control of ceramide levels by ceramide kinase: evidence from knockout animals and use of a novel potent inhibitor

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Background: The sphingolipid ceramide is an important regulator of cell biology (e.g. apoptosis, differentiation) and its levels therefore must be tightly controlled. Ceramide kinase (CerK) is a unique enzyme that specifically phosphorylates ceramide into the bioactive lipid ceramide-1-phosphate (C1P). Recent data from our laboratory have provided compelling evidence that CerK is a key regulator of both C1P and ceramide levels.
Methods: CerK-deficient (*Cerk*$^{-/-}$) Balb/C mice were generated as described in Graf et al. J Immunol 2008, 180:3457-66, and compared to control litermates in all studies. The diamino-benzothiazole derivative NVP-231 was synthetized as decribed in Graf et al. Mol Pharmacol 2008 74(4), in press. CerK activity assays using either fluorescently labeled ceramides or ^{32}P-ATP were described in Boath et al, J Biol Chem 2008 283:8517-26 and Rovina et al, Biochem J 2006 400:255-65, respectively. Growth and culture of COS-1 cells and bone marrow derived macrophages as well as Liquid Chromatography/Mass Spectrometry (LCMS) analysis was performed as decribed in Graf et al. J Immunol 2008, 180:3457-66.
Results: First, using a fluorescently labeled ceramide molecule to trace CerK activity we found that C1P is short-lived compared to other ceramide metabolites, thus fulfilling an essential criteria for signaling function. This suggested that CerK activity might be useful to dispose of excess ceramide. Then we profiled *Cerk*$^{-/-}$ mice and we found indeed that ablation of CerK not only decreases C1P levels but also leads to an increase in ceramide levels. Finally, we identified and characterized NVP-231, the first potent, specific and reversible CerK inhibitor. Consistent with the observations in *Cerk*$^{-/-}$ mice, preliminary experiments with NVP-231 suggest that CerK inhibition may have potential to regulate C1P and ceramide levels.
Conclusions: These results establish CerK as a novel, key regulator of ceramide levels and support further exploration of CerK inhibition as a rationale for the treatment of proliferative disorders.

Discovery of novel non-cyclam polynitrogenated CXCR4 coreceptor inhibitors

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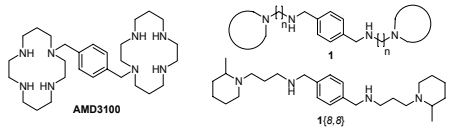
Background: CXCR4 and CCR5, chemokine coreceptors of the primary receptor (CD4) for the HIV cell fusion and entry, have been validated as targets for therapeutic intervention against AIDS. Bicyclams were the first non peptidic low molecular weight compounds with specific interaction with CXCR4, the most potent bicyclam being AMD3100 (IC_{50} = 1-10 ng/mL). However, it showed poor oral absorption and toxicity related to its high positive charge at physiological pH.
To overcome such problem, we designed a combinatorial library of tetraamines **1**, which preserve the main features of AMD3100: a) at least two nitrogen atoms on each side of the *p*-phenylene moiety, one in the benzylic position and the other(s) in a heterocyclic system and b) similar distances between such nitrogen atoms with those present in cyclam.
Methods: 19 compounds were initially selected by evaluating a series of molecular 2D and 3D descriptors. A PCA reduced the initial set of descriptors to 5 components which were used for the diversity selection. Anti-HIV activity (EC_{50}) and cytotoxicity (CC_{50}) measurements were carried out in MT-4 cells infected with HIV-1.
Results: The first subset of compounds showed EC_{50} in the range 0.9-18 µg/mL. A second subset of 17 compounds afforded 12 compounds presenting EC_{50} in the range 0.2-2.7 µg/mL. The third and final subset, covering up the total of 53 synthesized, was selected using QSAR techniques and ligand and structure-based drug design (using our CXCR4 and CCR5 modeled coreceptors). Among them, **1**{*8,8*} showed an EC_{50} value of 0.008 µg/ mL and a CC_{50} > 25 µg/mL, presenting nearly the same activity as AMD3100 but showing no cell toxicity at tested concentrations.
Conclusions: 1) A diversity oriented selection has allowed the synthesis of tetraamines **1** covering a broad range of activity values, useful for QSAR calculations. 2) This approach afforded compound **1**{*8,8*}, with an EC_{50} value of 0.008 µg/mL and a CC_{50} > 25 µg/mL. 3) Studies on the mode of action of compounds **1** showed specific inhibition of the CXCR4 coreceptor.



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Heparin as an inhibitor of cancer progression; the role of selectins

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Background: Heparin and low molecular weight heparin (LMWH) are widely used in cancer patients at risk of venous thromboembolism, which is a recognized complication of malignant disease. Recent clinical trials with LMWH and meta-analysis of earlier clinical trials with unfractionated heparin indicate that heparin affects also cancer progression. Meanwhile, heparin and LMWH were repeatedly shown to reduce metastasis in a variety of animal models. Heparin is a natural product, comprising a polydisperse mixture of highly sulfated glycosaminoglycan chains, only a fraction of which bind antithrombin.
Methods: Experimental metastasis study was performed in wild type as well as in P- and/or L-selectin deficient mice. Treatment with heparin or their derivatives was performed only shortly before or after the application of tumor cells. The extend of metastasis was evaluted after 28 days.
Results: We provided evidence that heparin has an additional biological activity which inhibits binding of P- and L-selectin to their natural ligands. Since also modified heparins without any anticoagulant activity were found to attenuate efficiently metastasis, the heparin effect on cancer progression may not be principally due to inhibition of coagulation. In fact, we have demonstrated that the selectins play critical roles during the hematogeneous phase of carcinoma metastasis in animal models. Natural selectin ligands on carcinoma cells were identified as mucins carrying sialylated, fucosylated and sulfated carbohydrate structures. Heparin treatment in wild type mice resulted in attenuation of metastasis similar to the one observed either P- and/or L-selectin in mice, indicating that inhibition of selectins significantly contributes to the anti-cancer effect. Inhibition of selectin-mediated interactions with carcinoma cells was found to be critical for early stages of a metastatic cascade. Most importantly, attenuation of metastasis can be achieved at clinically acceptable dosages. In addition, heparin contains also other biological activities including inhibition of heparanase, modulation of chemokines and growth factors activities.
Conclusions: The available evidence from preclinical analyses, together with the promising observations from clinical trials, merits further investigation of heparin as a potential anti-metastatic therapy.

According to registration: BASU Chinmay

Alterations in Female Reproductive Organs of Cyclic Rats Treated with Aqueous Extract of Moringa Oleifera Lam: Indication of Possible Role in Epithelial Ovarian Cancer

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Background: A hormonal etiology of epithelial ovarian cancer has long been suspected seeing its incidence menopausal age, and now the role of FSHR has also been demonstrated. Many ovarian cancer cell line express FSHR in them. In studies of the anticancer potential of plants used in folk medicine of Bengal, extracts of plants such as Oroxylum indicum, Moringa oleifera lam, Aegles marmelos could be considered as potential sources of anticancer compounds. Amongst them only Moringa oleifera lam has unique anticancer as well as hormonal property, which may or may not be attributable to isothiocyanate or glucosinolate that it contains. An animal experiment was planned to see effect of Moringa root extract in female reproductive system of mice.
Methods: 5 adult female mice of swiss strain of 30 gm each 2-control, 3-treated kept on stock diet, pellet, having nutritional value of 7 daysAn aqueous extract of the root was prepared according to a traditional method. In brief, 500 g of the root were placed in a container with 750 ml of water and boiled for 30 min. The preparation was left standing to cool and was then filtered. The filtrate, containing 66.7 mg root in 1 mL, was placed in small vials and kept in 4 degree C refrigerator until use. 1 ml of extract was used orally daily for 45 days.
Results: Attenuation of ovary and uterus was seen while mice tolerated the herb extrct well. There was reversal to pre estrus phase of adult mice as was revealed by PAP smear from vagina. In histology there was absence of follicles in comparison to control ovary. There was lesser amount of fibrosis in treated ovary.
Conclusion: Isothiocyanate of Moringa may inhibit proliferation of ovarian granulosa and other cells as it induces apoptosis via caspase-9 and -3 pathways, a family of calcium-dependent cysteine proteases. It may also act by inhibiting ERK1/2 and Akt survival signaling while simultaneously activating pro-apoptotic p38 and JNK1/2. There are reports to show that Moringa induced decrease in cerebral dopamine and norepinephrine which may influence and lower NGF and FSHR through central mechanisms. There is strong possibilty of using this agent in epithelial ovarian cancer and, as such, a cell line experiment is urgently necessary.

Serum and Alveolar Concentrations of Antibiotics during the Treatment of Ventilator-Associated Pneumonia

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Background: We assessed the serum and alveolar concentrations of antibiotics frequently used during the treatment of ventilator-associated pneumonia (VAP) in critically ill patients in order to optimize antimicrobial treatment in this particular population.
Methods: Various antibiotics (β-lactams, tobramycin, levofloxacin or linezolid) were administered in intermittent or continuous infusion to critically ill patients with VAP. At steady-state (after two days of therapy), blood samples were withdrawn from each patient and serum concentrations were measured by high-performance liquid chromatography. Simultaneously to blood sampling, antibiotic concentrations were determined in epithelial lining fluid (ELF) obtained from standardized mini-bronchoalveolar lavage, which is a reliable method for the measurement of alveolar drug concentrations.
Results: The 122 patients were of similar age (50-70 yrs), weight (60-80 kg) and creatinine clearance (60-100 mL/min). The antibiotic daily dose for each patient, administered in intermittent or continuous infusion, the serum and ELF concentrations and the ELF/serum concentration ratios appear in Table 1.
Conclusions: Serum and alveolar concentrations and alveolar diffusion of antibiotics from both different and similar classes exhibit wide variations in critically ill patients with VAP. Individual antibiotic dosages may be helpful to optimize the administration regimen of antibiotics and therefore PK/PD parameters ($T_{>MIC}$, C_{max}/MIC and AUC/MIC) during the treatment of VAP.

Table 1. Mean steady-state serum and ELF antibiotic concentrations

Antibiotic (dose/day)	*n*	Serum concentration (mg/L)			ELF conc. (mg/L)	ELF/serum conc. ratio
		Intermittent		Continuous		
		C_{max}	C_{min}	C_{ss}		
Cefepime 4 g	20	-	-	13.5	14.1	1.04
Ceftazidime 4 g	15	-	-	39.6	8.2	0.21
Ertapenem 1 g	15	30.3	0.8	-	9.4	0.32
Levofloxacin 500 mg qd	12	12.6	3.0	-	11.9	1.18
Levofloxacin 500 mg bid	12	19.7	7.7	-	17.8	1.27
Linezolid 600 mg bid	16	17.7	2.4	-	14.4	1.05
Piperacillin/tazobactam 12/1.5 g	10	-	-	25.4	12.7	0.46
Piperacillin/tazobactam 16/2 g	10	-	-	38.9	19.1	0.43
Tobramycin 7-10 mg/kg	12	22.4	-	-	2.6	0.12

Therapeutic effects of *Crocus sativus* (saffron) on respiratory diseases

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As indicated in ancient Iranian medical books *Crocus sativus* (Iridaceae) or saffron has therapeutic effects on respiratory diseases. Therefore in a series of experiments, the follwing effects of cumulative concentrations of saffron and its constituent, safranal was examined on tracheal chains of guinea pigs: relaxant, stimulatory effect on β-adrenoceptors, inhibitory effect on histamine (H_1) receptors and the inhibitory effects on muscarinic receptors

In precontracted trachea by 10 μM methacholine (roup 1) all concentrations of theophylline, extract and safranal showed significant relaxant effects compared to that of saline ($p<0.05$ to $p<0.001$). In precontracted trachea by 60 mM KCl (group 2) also theophylline, extract and safranal showed concentration dependent relaxant effects compared to that of saline ($p<0.05$ to $p<0.001$ for different concentrations except two low concentrations of safranal). However, in group 3 (precontracted incubated tissues with propranolol, chlorpheniramine and atropine by 60 mM KCl) the extracts of *Crocus sativus* showed a weak relaxant effect ($p<0.05$ only for highest concentration).

There were clear leftward shifts in isoprenaline curves obtained in the presence of only higher concentration of the extract in group 1(non incubated tissues) and it's both concentrations in group 2 (incubated tissues with chlorpheniramine) compared with that of saline. The EC_{50} (the effective concentration of isoprenaline, causing 50% of maximum response) obtained in the presence of both concentrations of the extract and safranal in group 1 and only in the presence of two concentrations of the extract in group 2 was significantly lower compared to saline ($p<0.05$ to $p<0.001$). The maximum responses obtained in the presence of both concentrations of the extract and safranal in group 1 were significantly lower than that of saline ($p<0.005$ for all cases).

EC_{50} histamine obtained in the presence of chlorpheniramine, all concentrations of the extract and safranal in all three groups (incubated trachea with: 1) indomethacin, 2) indomethacin, propranolol, and atropine and 3) indomethacin and propranolol) were significantly greater than those of saline ($p<0.05$ to $p<0.001$) except medium concentration of the extract in group 2 and its low concentration in group 3 (0.5 and 0.25 mg/mL respectively). The EC_{50} obtained in the presence of all concentrations of extract and safranal in group 2 were greater than group 1 and 3 ($p<0.05$ to $p<0.001$). Maximum response obtained in the presence of all concentrations of extract and safranal in group 2 were greater than those of group 1 and group 3 ($p<0.05$ to $p<0.001$).

The results of Inhibitory effects on muscarinic receptors showed clear parallel rightward shifts in methacholine-response curves obtained in the presence of atropine, 2 low concentrations of safranal and extract compared with the curves obtained in the presence of saline. The EC_{50} obtained in the presence of atropine, two lower concentrations of safranal and all concentrations of the extract was significantly higher than that of saline ($p<0.01$ to $p<0.001$). The maximum responses obtained in the presence of all concentrations of the extract was significantly lower than that of saline ($p<0.01$ to $p<0.001$).

These results showed a potent relaxant effect of saffron, a relatively potent stimulatory effect of the extract from *Crocus sativus* on ß $_2$-adrenoceptors, an inhibitory effect at histamine H_1 receptors and a possible inhibitory effect at muscarinicreceptors on tracheal chains of guinea pigs. The results also indicated that the safranal is, at least in part, responsible for the relaxant effect of *Crocus sativus*.

Different therapeutic effects of *Nigella sativa*

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In a series of studies the following effects of *Nigella sativa* on guinea pig tracheal chains and human airways were studied.
The results showed: (1) Aqueous and macerated extracts from *Nigella sativa* showed significant relaxant effect compared to saline ($p<0.001$). The extracts caused a non parallel rightward shift in methacholine concentration response curve and EC_{50} methacholine obtained in the presence of extracts were significantly greater than that of saline ($p<0.05$ to $p<0.001$). (2) plant extracts caused parallel right ward shifts in histamine concentration response curves obtained compared to saline. The EC_{50} histamine in the presence of extracts were significantly greater than slain ($p<0.05$ to $p<0.001$). (3) There was a leftward shift in isoprenaline concentration response curve in the presence of aqueous extract. (4) Different extracts showed significant relaxant effect on tracheal chains incubated with Chlorpheniramine and propranolol, contracted by methacholine ($p<0.01$ to $p<0.001$) but did not show any relaxant effect on tracheal contracted by KCl. (5) Aqueous extract caused a rightward shift in the $CaCl_2$ response curves and EC_{50} $CaCl_2$ hin the presence of axtract was significantly greater than the presence of saline ($p<0.05$). (6) However, thymoquinon, main constituent of the plant did not show any significant relaxant effect on tracheal chains. (7) Boiled extract of this plant caused significant increases in all measured pulmonary function tests (PFTs), ($p<0.05$-$p<0.001$) comparable to the effect of thophylline. (8) Two months administration of boiled extract also caused significant improvement in PFT values, respiratory symptoms, chest wheeze and drug usage in asthmatic patients. (9) Concentrations of extracts of the plant showed significant reduction of cough number ($p<0.001$ for all cases) which were significantly greater than that of codeine ($p<0.05$-$P<.001$).
Results showed a potent relaxant effect of *Nigella sativa* on tracheal chains, a relatively potent bronchodilatory effect on asthmatic airways and a potent antitussive effect.

Rediscovering antibiotics of alternative medicine: case of apitherapy

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Background: Antibiotic-resistant bacteria continue to be of major health concern world-wide. Since the use of antibiotics became widespread, bacteria have progressively developed resistance. Consequently, scientific efforts have been made to develop new compounds to be used beyond conventional antibiotic therapy. It has been proposed that the healing effect of bee products could be due to various physical and chemical properties. Hydrogen peroxide, volatiles, organic acids, flavonoids, beeswax, nectar, pollen and propolis are, among others, the chemical factors that provide antibacterial properties.
Methods: Bacteria of medical concern such as *Pseudomonas aeruginosa Escherichia coli* and Methicillin Resistant *Staphylococcus aureus* were subjected to the action of bee products; namely honey, propolis, royal jelly and bee venom. In the majority of cases, the minimum inhibitory concentrations (MICs) were obtained using the incorporation method. The MIC was determined by finding the plates with the lowest concentration of honey on which the strain would not grow.
Results: all bee products have shown antibacterial activity. Sensitivity of bacteria to bee products varies considerably within the product and the varieties of the same product. Propolis has been found to have the strongest action against bacteria. This is probably due to its richness in flavonoids. Antibiotic-resistant bacteria have been found to be sensitive to the action of hive products.
Conclusion:

- The frequent use of antibiotics has lead to the emergence of antimicrobial resistance.
- Honey and other bee products were subjected to laboratory and clinical investigations during the past few decades and the most remarkable discovery was their antibacterial activity.
- The emergence of antibiotic resistant bacteria, particularly methicillin-resistant *Staphylococcus aureus* (MRSA) has posed problems in the management of chronic wound infections. Many studies have shown that application of hive products to severely infected cutaneous wounds is capable of clearing infection from the wound and improving healing.
- Bee products are natural products; without adverse effects on tissues, they can be safely used on burns and inserted into cavities and sinuses to clear infection.

Neurotensin Agonists: Novel Analgesics with Synergy to Morphine

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Background: Neurotensin (NT) is a widely distributed neuropeptide in the central nervous system that modulates nociception at several different levels, but is associated with hypotension and hypothermia. NT exerts its effect through NT receptors, of which there are three known subtypes (NTS1, NTS2 and NTS3). Morphine is a μ-opioid receptor agonist that is commonly used for the treatment of many types of pain, but it is usually associated with side effects that can be serious. We hypothesize that selective NT receptor agonists may represent a novel class of analgesics and their use in conjunction with morphine will have synergistic properties with morphine which may reduce the dose administered and its side effects.
Methods: Studies were done to test for the use of a novel peptide analog of NT (NT69L) as a new class of analgesic drugs, acting through the NT receptors, alone and in combination with morphine. The antinociceptive activity of NT69L and morphine was studied in rats using the hot plate test to determine if there is synergism between the two drugs in reducing pain. The NTS2 receptor antagonist, levocabastine was used to determine the receptor subtype involved in the synergistic effect of NT69L and morphine.
Results: The administration of both NT69L and morphine resulted in a dose-dependent analgesic effect. Isobolographic analysis was used to study the antinociceptive interactions between the two drugs. The isobolographic analysis demonstrated that the combination of sub-analgesic doses of NT69L and morphine was synergistic in the hot plate test. Pretreatment with the NTS2 receptor antagonist, levocabastine, attenuated the synergistic effect of NT69L and morphine in the hot plate test.
Conclusion: The results provide preliminary data supporting the hypothesis that the synergistic combination of NT69L and morphine would improve the pharmacological treatment of pain while minimizing specific adverse effects of each of the drugs at a higher dose. Both NT receptors NTS1 and NTS2 are important for the synergisitic effect of NT69L and morphine.

Current Medical Countermeasures (Vaccines- Antibodies- Antibiotics) to Protect Humans from the Anthrax Bioterrorism.

BOUZIANAS DG

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Background: *B. anthracis* spore attacks through the US mail system have demonstrated their feasibility as a bioterrorism weapon. Vaccination appears to be the most effective and economical form of mass protection, however, the current vaccines have drawbacks that justify the immense efforts for the development of improved treatment modalities. This review summarizes the current human approaches developed mainly since the 2001 events against inhalational anthrax.
Methods – Results: The increased research activity has led to a huge expansion in the existing literature. The present work is based on an extensive review of the international literature. Combination of postattack prophylactic vaccination with antibiotic therapy is the most effective strategy. Current approaches against anthrax toxins are focused on agents that affect crucial steps of the intoxication process. High-affinity toxin-specific monoclonal antibodies have a significant clinical effect, if they are administrated rapidly. While effective antibiotics, antitoxins and vaccines are available, concerns over their safety and effectiveness have driven the development of 2nd and 3rd generation products that act rapidly and with minimal adverse effects. Protective antigen (PA) is the principal immunogen of the 1st generation licensed vaccines. A 2nd generation vaccine is based on highly purified recombinant PA (rPA) and is likely to receive licensing approval. The 3rd generation vaccines aim to enhance the efficacy of the previous vaccines. They would ideally be given via the oral, nasal or dermal routes for delivery of rPA in a single dose facilitating stockpiling and mass vaccination programs. DNA vaccination could form the basis for multiagent vaccine development. The development of novel agents is hampered by the difficulty in demonstrating effectiveness in humans.
Conclusions: Treatment response to a deliberate release of *B. anthracis* spores includes the prompt administration of antibiotics. There are concerns for the available antitoxins and vaccines over their effectiveness and toxicity. Despite the intensive anthrax research, there has been as yet no real progress. The huge efforts are expected to provide an array of novel protective agents and their balloning list reflects the emergency of the global community to combat the anthax threat.

Cefepime Neurotoxicity in Perspective

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Background: Cefepime, a widely used antibiotic, is more effective than other cephalosporins against many Gram positive and negative microorganisms, and has a main role in the treatment of febrile neutropenia. Its safety profile was considered adequate, but increased mortality was recently found with its use. Most common neurological side effects are somnolence, disorientation, hallucinations, and epileptic seizures. Jallon et al. firstly reported on 19 patients who developed a confusional state that reverted after cefepime discontinuation. Cefepime?s neurotoxicity clearly results from its accumulation in the central nervous system (CNS). Excessive dosage and impaired renal clearance are important risk factors, as well as uremia, extreme ages, and meningitis. Our group employed three studies regarding clinical, EEG, and pharmacological issues of cefepime encephalopathy
Methods: We previously published a series of seven patients with cefepime encephalopathy. Thereafter, we studyied the renal failure as a risk factor for this clinical entity, applying the Cockcroft-Gault formula to measure glomerular filtration rates (GFR). The third study, now ongoing, looks for detecting the incidence of cefepime encephalopathy in different grades of renal failure. When neurological manifestations during cefepime treatment are detected, we record an EEG and measure cefepime blood levels as soon as possible. Events are classifyied according to Naranjo?s algorhythm, and renal impairment as absent, slight, moderate, severe, and terminal.
Results: In the first study, our patients used cefepime doses between 4 to 8 g/day. Somnolence, agitation, or myoclonic jerks developed after 2 to 9 days after starting treatment. Almost all patients had renal failure, and only one had cefepime dosage adjusted for renal function. The EEG pattern described by Jallon et al. were present in all patients. In the second study, we included 498 patients, 111 with renal failure. Five patients (1%) had encephalopathy, all in the renal impairment group. We also found that encephalopathy?s incidence increases with decreasing clearance rates. Indeed, in our present study, we hope to show that cefepime?s dosage is so important than renal function to the pathogenesis of cefepime encephalopathy.

Poor permeability of blood brain barrier for creatine: Autonomous brain synthesis of creatine, and consequences for creatine deficiency syndromes

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Abstract :

In mammals, creatine (Cr) is taken up from the diet, or can be synthesized endogenously by a two-step mechanism involving L-arginine:glycine amidinotransferase (AGAT), which yields the intermediate guanidinoacetate (GAA), and guanidinoacetate methyltransferase (GAMT), which converts GAA to Cr. Cr is distributed through the blood and is taken up by cells with high energy demands through a specific Cr transporter, SLC6A8. It was thought for a long time that most, if not all, of the Cr needed by the brain comes from the periphery through blood brain barrier (BBB). However, our recent work has shown that AGAT and GAMT are expressed in CNS and that brain cells in vitro synthesize their own Cr. SLC6A8 is also expressed in CNS, but not in astrocytes, particularly in their feet sheathing microcapillaries at BBB. These data suggested that BBB has a limited permeability for Cr, and that CNS might depend more on its own autonomous Cr synthesis than on Cr supply from the blood.

The brain is the main organ affected in patients suffering from Cr deficiency syndromes caused by either AGAT, GAMT or SLC6A8 deficiencies, which all three are characterized by an absence, or a severe decrease, of Cr in CNS, as measured by magnetic resonance spectroscopy. Because SLC6A8 is present in microcapillary endothelial cells, AGAT and GAMT deficient patients can be treated with oral Cr supplementation. However, due to the absence of SLC6A8 in the surrounding astrocytes, very high doses of Cr must be used, and the replenishment of cerebral Cr takes months and results only in the partial restoration of the cerebral Cr pool. Cr supplementation of SLC6A8 deficient patients is inefficient to restore cerebral Cr levels.

Low-dose steroids in critically ill patients: Who should be treated and when?

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Ongoing and severe systemic inflammation affecting critically ill patients may cause adrenal insufficiency and steroid resistance. As this clinical entity is difficult to diagnose, many studies on low-dose corticosteroid therapy used inclusion criteria based on the underlying disease, clinical symptoms, time windows after onset of the disease, and biochemical testing. Septic shock is without doubt the best known disease with severe systemic inflammation and related adrenal insufficiency. In many studies, persisting septic shock after fluid and vasopressor resuscitation became the main inclusion criteria. Other target groups in low-dose corticosteroid trials were patients with ARDS, acute lung injury (ALI) due to pneumonia, cardiac surgery with cardiopulmonary bypass, acute pancreatitis, or trauma.

Target group septic shock
Septic shock and low-dose corticosteroid therapy have been extensively investigated during the last decade [1-6]. The main finding supported by all studies is that hydrocortisone 200 to 300 mg per day ***accelerates shock reversal***, i.e. reduces the time on vasopressor therapy. Despite different inclusion criteria of septic shock (i.e. early vs. late septic shock, hypotension and poorly responsive to fluid and vasopressors resuscitation vs. the broader definition given by the ACCP and the SCCM), this finding is consistent and supported by the most recent trial, the large European CORTICUS study [1-6]. In the latter clinical trial, improvement in cardiovascular physiology did not translate in improved survival as it has been demonstrated earlier in the Annane study [1,2]. In addition, adjustment of the appropriate target group by means of corticotropin tests did not make a difference in the CORTICUS trial [1]. This is in marked contrast to the results of the Annane study, in which the benefit of hydrocortisone and fludrocortisone was found in the target group with a blunted response to corticotropin as defined by post-corticotropin cortisol increase of ¡Ü9 µg/dl. The reasons for the differences found in the two clinical trials remain to be discussed. First analyses revealed that patients in the Annane study were more severely ill (SAPS II: 59 vs. 49 pts.), had early septic shock (time window of inclusion: 8 vs. 72 hrs.), had more severe arterial hypotension and higher vasopressor doses at inclusion, did not respond to volume therapy for at least one hour, and had more pneumonia as underlying infection. The most important difference was the 28-day mortality of the placebo group which was 61% in Annane study and 31.5% in the CORTICUS trial. A post-hoc analysis of patients in CORTICUS who had a systolic blood pressure that persisted below 90 mm Hg at 1 day after fluid and vasopressor resuscitation (n=126) showed a rate of death of 56.1% in the placebo group and an absolute reduction in mortality of 11.2% in the hydrocortisone group. These results that are very similar to those reported by Annane [2]. CORTICUS patients who received the study drug within 12 hours after baseline did not show any significant differences in outcome when compared with patients who have received the study drug later. The results of the CORTICUS trial led to the new recommendation of the surviving sepsis campaign that only patients with septic shock poorly responsive to fluid resuscitation and high-dose vasopressor therapy should receive hydrocortisone therapy, but not all patients fulfilling the broader definition of septic shock given by the ACCP and the SCCM. The optimal time window for initiation of hydrocortisone in septic shock remains uncertain.

Target group ALI-ARDS
Ongoing systemic inflammation associated with excessive fibroproliferation in persistent ARDS has been proposed as another indication for corticosteroid therapy. Methylprednisolone treatment exceeding the dose of corticosteroid replacement therapy in the studies on septic shock by a factor of two improved pulmonary function and reduced multiple organ dysfunctions by profound immunomodulation of the persistent inflammatory process [7, 8]. The large-scale trial of the ARDS network, however, did not support this intervention [9]. Despite significant and substantial improvements in pulmonary physiology associated with a higher number of ventilator-free days and ICU-free days at day 28, the use of corticosteroid did not result in a lower mortality at day 60 after randomisation. An increased rate of return to assistant breathing associated with muscle weakness has been discussed as main reason why improved physiology did not translate in improved outcome. A post-hoc analysis revealed that patients with ARDS of 14 to 28 days duration (n=48) had even increased 60-day mortality when they were assigned to the corticosteroid group whereas a trend to improved survival was seen in the group recruited between day 7 and 13 after onset of ARDS [9]. It has been criticized that substantial imbalances at baseline in the latter post-hoc analysis group were present favouring the placebo group.

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The design of drugs which do not create resistance: folding inhibitors

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Conventional protein inhibitors cap the active site of their targets, preventing the binding of the substrate. An alternative approach has been recently developed to block the activity of proteins and consists in preventing their folding to the native, biologically-active conformation. It is based on recent understandings on the overall folding mechanism of proteins, and in particular on the observation that some protein segments display residual native structure already in the denatured state of most proteins, and that the same segments often participate to the formation of the transition state, as shown by protein-engineering experiments. The theoretical picture which emerges is that folding is guided by Local Elementary Structures (LES) and stabilized by few, highly conserved ("hot") amino acids. Docking of the LES give rise to the transition state and to the postcritical folding nucleus (FN), which inevitably grows into the native state. Intervening a folding reaction can, in principle, be achieved by interacting the polypeptide chain with peptides (called p-LES) whose sequence is identical to those of the LES that define the FN of the target protein. As the concentration of p-LES increases, the protein may nucleate by the assembly of the protein chain with peptide LES, leading to a nonproductive folding. This can be viewed as changing the folding from a unimolecular reaction to a bimolecular reaction.
These are two important advantages of these non-conventional (folding) inhibitors with respect to conventional (active-site centered) ones. First, their molecular structure is suggested directly by the target protein. One needs not to design or optimize anything, just find the LES of the protein to be inhibited, because the design has been performed by evolution through a myriad of generations of the organism that expresses the protein. Moreover, the probability that the protein can develop resistance through mutations is much smaller than in the case of conventional drugs. In fact, a folding inhibitor binds to a LES, and a protein cannot mutate the amino acids of a LES under risk of denaturation.
The above strategy has been applied to HIV-1 Protease, an enzyme which plays an essential role in the lifecycle of the virus. Consequently, its inhibition can control AIDS. The HIV-1 PR is an homodimer, each monomer containing 99 amino acids. It reaches the native conformation following a three-state mechanism in which each monomer folds independently of each other and afterwards they dimerize.
We have identified the segments containing the residues 23-33 and 83-92 as those associated with the LES of the monomer, the segment 83-92 being the one that becomes more structured in the early folding events. Experiments in silico[1,2], in vitro[3] (enzyme) and in infected cells[4] (virus, also multi-drug resistant virus) have shown (see also 5) that peptides with identical sequence to the segment 83-92 (p-LES(83-92)) are able to inhibit enzymatic activity by inhibiting folding, as testified by circular dichroism experiments[3]. Furthermore, "long-term" studies of pharmacological pressure conducted by passing in PBMC of a wild-type virus in the presence of p-LES(83-92) or Atazanavir (ATV) have shown the very high genetic barrier to mutations displayed by the LES peptide. In fact, genotipic sequenceing[6] showed that p-LES (83-92) did not select for any mutation leading to resistance, since the pattern of mutations at baseline and after 9 months of in-vitro pharmacological pressure were just alike. On the other hand, ATV selected for primary and/or secondary mutations. The transferibility of the folding-inhibition strategy to other proteins testify to the universality of the folding-inhibition scenario for the design of leads of drugs which are unlikely to generate resistance[7,8].
1. Broglia RA et al (2005) Protein Sci. 14:2668-2681 **2.** Broglia RA et al. (2007) La rivista del Nuovo Cimento 29:1-119 **3.** Broglia RA et al (2006) Proteins 62:928 **4.** Rusconi S et al (2007) Procs. Of the International School of Physics "E. Fermi", Course CLXV on Protein Folding and Drug Design, IOS Press, Amsterdam, 293-299 **5.** Broglia et al (2008) Curr. Opin. Struct. Biol. 18:60-66 **6.** Ferramosca S et al., XVII International HIV drug resistance workshop, basic principles and clinical implications, June 10-14, 2008 Sitges, Spain (see http://merlino.mi.infn.it/publications/articles2/asbtract_2008.pdf) **7.** Broglia RA et al. (2007) Proteins 67:469 **8.** Caldarini M et al (2008) Proteins
(in press; see http://merlino.mi.infn.it/publications/articles2/lysozyme12fig.pdf)

Novel targets and magic bullets for treating cardiovascular disease

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Cardiovascular disease represents a major clinical problem affecting a significant proportion of the world's population and it remains the major cause of death in the EU and the rest of the Western world. Furthermore, the burden on healthcare systems is increasingly high; the overall cost of cardiovascular disease to the EU economy is estimated to be in excess of 192 billion Euros per year. The majority of therapies currently available for the treatment of cardiovascular disease do not cure the problem but merely treat the symptoms. Furthermore, many cardioactive drugs have serious side effects and have narrow therapeutic windows that can limit their usefulness in the clinic. Thus, the development of more selective and highly effective therapeutic strategies that could cure specific cardiovascular diseases would be of enormous benefit both to the patient and to those countries where health care systems are responsible for an increasing number of patients. There is increasing evidence to suggest that targeting the cell cycle machinery in cardiovascular cells (e.g. cardiac myocytes, vascular smooth muscle cells (VSMCs), endothelial cells) provides a novel approach for the treatment of certain cardiovascular diseases, including post-infarct heart failure, restenosis, in-stent stenosis and bypass graft failure. It has been demonstrated that certain cell cycle molecules that are important for regulating terminal differentiation in cardiac myocytes (e.g. cyclins, cyclin-dependent kinases [CDKs], CDK inhibitors, E2F transcription factors) can be targeted to reinitiate cell division and repair in the myocardium post-infarction. Furthermore, cell cycle molecules that control excessive VSMC proliferation in disorders such as restenosis, in-stent stenosis and bypass graft failure have also been targeted effectively in recent laboratory and clinical studies. The results of these studies illustrate the exciting possibility of targeting components of the cell cycle machinery to develop magic bullets to improve cardiac function and prognosis for patients with heart failure and for patients with atherosclerosis.

A Therapeutic Conundrum: Low Molecular Weight Heparins in Patients with Kidney Dysfunction.

BROPHY DF

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Background: Patients with chronic kidney disease (CKD) are frequently viewed as being at high risk for bleeding. In point-of-fact however, CKD patients not only maintain a bleeding tendency but also exhibit a high frequency of thromboembolic disease. Indeed, cardiovascular disease is the leading cause of death in CKD patients. Therefore, clinicians are frequently confronted with choosing the most appropriate antithrombotic therapy in CKD patients undergoing percutaneous coronary intervention. Clinical trials have shown that low molecular weight heparins (LMWH) have greater efficacy and less adverse effects compared to unfractionated heparin (UFH). However, CKD patients present a special circumstance such that LMWHs carry an increased risk of adverse bleeding in such patients compared to those with normal renal function. This presentation will provide perspective on the clinical use of LMWH in CKD patients.
Methods: A Medline literature review was conducted to identify primary literature describing the efficacy and risk of adverse bleeding when UFH and LMWH drugs were used at therapeutic doses in CKD patients.
Results: In major clinical trials, enoxaparin has been associated with increased bleeding rates. A recent meta-analysis carefully described the available data for all LMWH products in non-dialysis-dependent CKD patients with respect to bleeding. n the primary analysis, 4971 patients with a creatinine clearance < 30 mL/min had an increased risk of bleeding compared to those without renal insufficiency (5.0% versus 2.4%; odds ratio 2.3, [95% CI 1.2 to 4.3], p=0.01). In a secondary product-based analysis of these same data, enoxaparin use increased the rate of major bleeding to 6.0% in CKD patients compared to 2.4% in non-CKD patients (odds ratio 2.6, [95% CI 1.2 to 4.3]). The likelihood of a major bleeding event increased further when the enoxaparin dosing regimen was considered. Safety data for other LMWH is difficult to ascertain because of the relative few available data.
Conclusions: These data suggest that non-adjusted enoxaparin dosing may lead to serious bleeding in CKD patients. The appropriate enoxaparin dose reduction has yet to be confirmed. The use of UFH is prudent in patients with advanced CKD.

Epinephrine Vasoconstrictor Drug-Drug Interactions Revisited

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Drug-Drug interactions with regard to epinephrine vasoconstrictor local anesthetic formulations have been vastly overstated in the past. Such purported drug interactions include tricyclic antidepressants, non-specific beta blockers and cocaine. These supposed but mistaken interactions are widely published in many Dental Pharmacology Texts and have established problematic clinical considerations negatively influencing pharmacologic patient therapy. A portion of the rationale for such purported drug interactions include poorly designed studies and inapplicable case reports. The major misconceptions are due to misunderstandings of adrenergic pharmacology. Lack of understanding with regard to epinephrine's beta two receptor's influence upon blood pressure dynamics and the difference between alpha adrenergic local versus system effects are problematic. A misunderstanding of the positive attributes of local anesthetic vasoconstrictor action and limited knowledge of sympathetic activation and the actions of endogenous norepinephrine among dental clinicians has contributed to this problem and resulted in the misuse of pharmacotherapeutics. The lack of toxicity is further influenced due to epinephrine's exceedingly short half-life. To add to the above, the mechanism of tricyclic antidepressant pharmacology is not common knowledge among dental clinicians and even such simple drug-drug interactions as the additive drug-drug interaction between cocaine and other local anesthetics is overlooked. Furthermore, many recent human studies which demonstrate the relative safety of epinephrine vasoconstriction will also be discussed. In conclusion, an evaluation of potential drug-drug epinephrine vasoconstrictor interactions is important with regard to clinical dental care and appears to limit fears regarding several noted but highly suspect previously accepted drug-drug interactions.

Magic bullets for the treatment of inflammatory bowel disease – yet to come?

BRUNNER M

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Background: Inflammatory bowel disease (IBD), an immune-mediated chronic intestinal condition, encompasses two idiopathic inflammatory diseases of the intestinal tract: Crohn's disease and ulcerative colitis. Currently, medical treatment for IBD aims at induction and maintenance of remission. Established therapies, such as 5-aminosalicylic acid compounds, corticosteroids, immunomodulators or calcineurin inhibitors, however, lack specificity or effectiveness and might cause significant long term side effects.
Recent advances in the knowledge of pathogenesis and immunology of IBD has led to the development of novel therapies directly targeting specific aspects of the inflammatory process, such as cytokines and receptors involved in T-cell activation, selective adhesion molecule blockers, anti-inflamatory cytokines, modulators of the intestinal flora or monoclonal antibodies. The first monoclonal TNFα antibody, has been successfully introduced into clinical practice for the treatment of IBD approximately 10 years ago. Most other novel therapies are undergoing different stages of clinical evaluation.
Conclusions: Although during the last years progress has been made in both defining the mechanisms underlying the development of inflammatory bowel disease and expanding the spectrum of effective therapies, it might be too early to know, whether the currently tested compounds will be routinely employed in IBD treatment. It seems rather unlikely, however, that one single drug will prove to be a magic bullet.

Transcriptional Changes Induced by Imatinib and Nilotinib in the Chronic Myelogenous Leukemia (CML) Cell Line K562

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Background: Nilotinib is a selective bcr-abl tyrosine kinase inhibitor that is 30-fold more potent than Imatinib in vitro. To examine the molecular and functional effects of Nilotinib and Imatinib we performed gene expression and functional analyses in K562 cells following in vitro treatment with the two tyrosine kinase inhibitors. Particular emphasis was put on 1539 genes which we found to be differentially expressed in primary CD34+ cells from patients with CML in chronic phase in comparison to CD34+ cells from normal bone marrow (Diaz-Blanco et al., Leukemia 2006).
Methods: Affymetrix U133A 2.0 microarrays covering 21.722 probe sets were used to analyse the gene expression profile of $5x10^7$ K562 cells after 24h in vitro treatment with Imatinib (0.5 µM) or Nilotinib (0.05 µM) (half maximal inhibitory concentration). FISH analysis confirmed the K562 cell line to be BCR-ABL positive. Gene expression data of the treated cells were compared with the data of untreated cells. In addition, proliferation (MTS Assay, Promega), apoptosis (Cell Death Detection ELISAPLUS, Roche) and cell cycle (FITC BrdU Flow Kit, BD Pharmingen) assays were performed.
Results: Looking at those 1539 differentially expressed genes in K562 cells which distinguish patients with CML from healthy donors, we found that Imatinib led to a significant downregulation of 187 and upregulation of 45 genes. In general, the effect of Nilotinib with regard to the number of genes affected and degree of suppression was more pronounced resulting in the downregulation of 418 and upregulation of 41 genes. Of note, genes affected by Nilotinib included all genes altered by Imatinib such as those related to bcr-abl signalling. Downregulation of genes involved in cell cycle was only observed following Nilotinib exposure. The stronger effect of Nilotinib is in line with the results of cell cycle experiments showing that Nilotinib exposed cells had the lowest proportion of actively cycling cells. The proportion of apoptotic K562 cells was 5.5 fold greater following treatment with Nilotinib in comparison to Imatinib after 24 hours.
Conclusion: Nilotinib is apparently more potent than Imatinib with regard to the number of genes downregulated and the degree of their suppression. Many of the suppressed genes are associated with bcr-abl signalling and cell cycle.

Authors' disclosure statement: Imatinib and Nilotinib were provided by Novartis. The work was supported by Leukaemieliga e. V.

according to registration: Bugelli Cainelli Gebara!

Immunogenic and Adjuvant Activities of *Bordetella pertussis* proteins

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Background: Adjuvants are essential components to enhance the immunogenicity of vaccine antigens. Extracts of bacterial origin are well known and widely used immunomodulatory substances. *B. pertussis* is a promising candidate since it produces several components acting on the immune system of the host.
Methods: The proteins were purified by anion exchange chromatography (Hitrap Q Sepharose HP) followed by extraction from a preparative gel (SDS-PAGE), and were further analysed by mass spectrometry and tested in mice. Female BALB/c mice, 4-6 weeks old were subcutaneously injected 2 times at weekly intervals with the proteins (1µg/0.1ml/mouse) alone or mixed with DPT (Diphtheria-Pertussis-Tetanus) vaccine formulated without aluminum hydroxide (NA-DPT), (2µl/mouse). DPT vaccine containing aluminum hydroxide (DPTBut) was used as control (2µl/mouse). Non-immunized mice (saline solution injected) were used as control. Blood was collected 1 day before and 20 days after the first immunization, and mice were challenged by intracerebral route with live *B. pertussis*. Sera were assayed for antibodies and isotypes. ELISA was used for all evaluations. Statistical analyses included one-way ANOVA followed by Dunnet's multiple comparison tests considering $p<0.05$, and χ^2 test, with Yates correction.
Results: We have purified two *B. pertussis* proteins, and identified by mass spectrometry as the 73 kDa N-terminal α-domain of BrkA autotransporter protein and the Cpn60/60 kDa chaperonin.
Immunizations with 73k+60kDa+NA-DPT elicited a significant increase ($p<0.01$) of the antibody response against pertussis, and of pertussis specific IgG1 and IgG2a, when compared to DPTbut immunization. The former group have also showed a better protection rate (42%) against the challenge than the latter (27%).
Conclusions: Our results suggest that mouse immunization with both proteins mixed with NA-DPT could stimulate Th1 and Th2 cells, induce significant higher levels of IgG1 and IgG2a antibodies, and mediate a partial protection against the challenge. Therefore, these proteins are good candidates to be exploited as adjuvants for inclusion in vaccination protocols.
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Online method for measuring of the activities of antibiotic efflux pumps in *Escherichia coli* and *Pseudomonas aeruginosa*

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Background: Multidrug resistance (MDR) pumps, decreasing intracellular concentrations of antibiotics are major causes of resistance in opportunistic pathogens such as *Pseudomonas aeruginosa*. This phenomenon ensures the viability of bacteria under high concentrations of antibiotics and complicates the treatment of infected patients. Fast methods are needed to determine the efficiency of drugs inactivating MDR pumps for the successful treatment of patients.
Methods: Aerated suspensions of *Escherichia coli* and *P. aeruginosa* cells were studied in thermostated reaction vessels, and changes in the extracellular concentration of indicator lipophilic cation tetraphenylphosphonium (TPP^+) was monitored using selective electrodes. Tetracycline was used as the model antibiotic, and, phenylalanyl arginyl β-naphthylamide (PAβN), reserpine and chlorpromazine were used as inhibitors of the different MDR pumps.
Results: Depending on the outer membrane permeability, membrane voltage and activity of the MDR pumps, *wt* cells and the main MDR pump mutants of *P. aeruginosa* and *E. coli* accumulated different amounts of TPP^+. Addition of tetracycline and the pump inhibitors caused detectable alterations of the amount of TPP^+ accumulating. Using the outer membrane-permeabilizing and the plasma membrane depolarizing compounds, activity of the pumps, affinity of the substrates and efficiency of the inhibitors were evaluated.
Conclusion: Online monitoring of TPP^+ fluxes across the bacterial envelope can be applied for studies of the activity of MDR pumps in *E. coli* and *P. aeruginosa*, for determination of the pump selectivity to substrates and for evaluation of the efficiency of pump inhibitors.

Red Blood Cell Membrane Fatty Acid Analysis in Never-Medicated First-Episode and Chronic Medicated-Schizophrenic Patients

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Background: Reduced n-3 and n-6 polyunsaturated fatty acids (PUFAs) content in red blood cell (RBC) membranes and abnormal membrane phospholipid metabolism were repeatedly implicated in the etiology of schizophrenia.
Methods: Gas-chromatography analysis of fatty acids content in RBC was performed in the group of never-medicated first-episode schizophrenic patients, chronic medicated patients and healthy controls. Differences between group means were investigated using ANCOVA. Group, sex and smoking status were used as predictors, while covariates were body mass index (BMI) and age. The level of statistical significance was set to 0.01.
Results: Three groups of subjects significantly differed in total n-3 fatty acid, 22:6n-3, 22:5n-3 and 22:0 RBC content, delta-9-desaturation (D9C18) index, peroxidizability index (PI), double bond index (DBI), and DBI/PI ratio. BMI was significantly correlated to average chain length (ACL), D9C18 index and PUFA/monounsaturated fatty acid ratio. First-episode patients had significantly higher total saturated fatty acid (SFA) content, particularly 18:0, lower total PUFAs content, particularly 18:2n-6 and 22:5n-3, lower D9C18 index, PUFA/SFA and DBI/PI ratios compared to controls. The differences between chronic medicated-patients and controls showed similar pattern. Only minor but highly significant differences were found between two groups of schizophrenic patients (22:0, 22:5n-3).
Conclusions: 1) The results confirmed a disturbance of lipid homeostasis in RBC membranes as intrinsic feature of schizophrenia. 2) Reduced total n-3 FAs and total PUFAs content, reduced PI, DBI and D9C18 index, and higher total SFA and 18:0 content were found regardless of illness duration and antipsychotic treatment. 3) Nicotine usage was not a significant predictor of the FAs composition in RBC membranes of investigated subjects. 4) Drugs affecting phospholipid metabolism and providing recovery of lipid homeostasis in cellular membranes might have beneficial effects in schizophrenia.

Conceptual basis for cancer treatment: from single drugs to kits with serial programmed actions of multiple substances-drugs

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Background: Well-known paradoxical effect that both high-dose estrogens and anti-estrogens cause tumor regressions. In this case important is an understanding of the properties of a tumor. Aims: 1) To study of the influence of the same hormonal environment on tumor growth and character of change of the receptors level in tumors with different hormone sensitivity.
Methods: Concentration femtomoles/mg protein (fM/mg) of estrogen receptor (ER) was determined in transplanted mice mammary tumors with intensive reproduction mode (2-6 parturition without lactating) (n=20) and virgin (n=20). The levels of ER were determined by means of the dextran-coated charcoal technique. Mice of line C3H/Sn (n=69) contained in cages on 5 female +2 male (normal reproduction) in a room with daylight. Virgin female mice (n=47).
Results: The data provides both estrogen-positive and –negative tumors that actually are evoked from the same tissue, so that one can determine at what level(s) the hormone sensitivity or dependency exists. The widely accepted hypothesis that the interaction of estrogen with its cellular receptors determines the hormone dependency of mammary tumors should now be challenged on the basis of the following observations: a) tumors occuring in experimental animals with permanent high estrogen levels are receptor-positive(~20-100fM/mg) and with low estrogen levels (<2fM/mg) are receptor-negative; b) tumors regrowing after complete or partial regression as a result of endocrine ablation or hormone administration are no considered to be autonomous or hormone dependence but environment dependence; c) growth of a tumor at cyclically changing hormonal level leads to heterogeneity of mammary tumors that complicates hormone therapy.
Conclusions: 1) Series of parameters of a tumor such as the invasiveness, heterogeneity and others are consequence of adaptic properties of tumoral cells. 2) Ability of tumoral cells to adapt for change of a surrounding microenvironment answers on a question on a paradoxicality of a hormone therapy. 3) High sensitivity of tumors to change of steroids concentration (10^{-9}M) discovers an epigenetic path of redifferentiation a tumor oncogenome i.e. multiple-stage therapy.

Pro-Atherogenic and Pro-Inflammatory Alterations in Mononuclear Cell Populations Induced by Oxidized Low Density Lipoprotein (oxLDL) and High Glucose Levels in Type II Diabetic Patients. Anti-Inflammatory Drugs, an Alternative?

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Background: Type II diabetes mellitus is a risk factor in the development of atherosclerosis. Two factors are central to the effect of diabetes, oxidative modification of LDL and high glucose concentrations. We hypothesized that mononuclear cells from diabetic patients in the presence of oxLDL and high glucose levels undergo pro-inflammatory alterations, which would enhance the development of atherosclerotic plaques. Therefore, our aims were: 1) To measure the induction of necrosis and apoptosis in mononuclear cells from diabetic patients, 2) to measure the release of pro-inflammatory interleukins in stimulated cells, and 3) to analyze the possible use of meloxicam as an alternative treatment.
Methods: This study included cells from 140 diabetic patients and 105 controls. The individuals were grouped by age, sex and obesity for the measurement of necrosis and apoptosis; 15 diabetic and 15 controls for quantification of interleukins; and 20 patients and 20 controls to study the effect of meloxicam (7,5 mg/day/30days). The results are presented as the mean ± SD of at least three independent experiments (a value of $p < 0,05$ was considered as significant, t-test).
Results: Cell necrosis and apoptosis was increased in mononuclear cells from obese, diabetic patients older than 50, without significant sex-related differences. Moreover, the release of pro-inflammatory interleukins, in particular those related with the development of atherosclerosis, was also increased in diabetic cells. Interestingly, meloxicam treatment decreased necrosis, apoptosis and release of pro-inflammatory interleukins from diabetic mononuclear cells.
Conclusions: Some of the pro-inflammatory effects observed when diabetic cells are subjected to the synergistic action of oxLDL and high glucose concentrations, such as necrosis, apoptosis and interleukin release, are reduced by treatment with meloxicam. Acknowlegment: FONDECYT 1040977, DIP-UCSC, Prof. Max Bachem.

Tacrolimus: a highly effective new therapy for chronic glomerular diseases?

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Abstract:

Tacrolimus is a potent immunosuppressive agent that has been demonstrated to be superior to cyclosporine after renal transplantation as an effective and safe anti-rejection drug. Based on the excellent outcomes in the transplant population, the use of tacrolimus has been extended to patients with a variety of different chronic glomerular diseases that are immune-mediated, such as steroid resistant nephroses and lupus nephritis, to name a few. The aim of this presentation is to critically appraise data related to the safety and efficacy of tacrolimus in adults and children with immune mediated renal diseases, outside the transplant setting. New evidence discussing the potential benefits and side-effects of long-term tacrolimus use (such as subclinical nephrotoxicity) will be discussed, so as to allow the audience to make an evidence-based assessment on whether tacrolimus is a reasonable strategy to treat patients with chronic glomerulonephritis.

The two main problems in evaluating resistance to antiparasitic drugs in populations of naturally infected hosts: efficacy variability and cut-off value for resistance

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Background: Variability of parasite/host response to antiparasitic drugs is large in field conditions depending on drug and galenic formulation. Resistance to anthelmintics has been well documented in sheep and goats, partly in cattle and less frequently in man. We dispose of different evaluations of efficacy for anthelmintics, none of them being a golden standard. We do not really know up to what level of efficacy we consider that we are facing a resistance phenomenon. In that respect it has been considered that less than 90% (horses) or 95% (ruminants) efficacy was the limit for stating on resistance. We are in great need of evaluation of efficacy of drugs and cut-off value for resistance.
Methods: Since distribution of efficacy values is not known and is clearly not a Gaussian distribution, we propose a bootstrap confidence evaluation, using a freeware we constructed (Bootstreat available on demand or on internet). The bootstrap evaluation is based on different evaluation formulas for efficacy (before /after treatment with and without untreated control, after treatment in treated and controls, either using arithmetic or geometric means). The evaluation of cut-off for resistance is a completely open problem. We propose a two steps' method: transformation of individual efficacy data that results in a Gaussian distribution and then when efficacy is not real we suppose that Gaussian distribution is acceptable and progressively with increasing doses and /or efficacy, there is a departure from such a distribution. We use data from anthelmintic treatments (tetramisole, macrocyclic lactones, benzimidazoles) in cattle or sheep.
Results: Bootstrap results indicate that usual procedure for estimating efficacy are not accurate since confidence intervals reach sometimes more than 20% of the mean. The cut-off values are highly dependent on drug/dose (example in tetramisole) and galenic form used (injectable or per os ivermectin). We propose a strategy for evaluating resistance in the field.

Botulinum Toxin in restrictive strabismus

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SUMMARY

The restrictive strabismus is the most frequent sequel in Thyroid Orbitopathy

Objective. We want to determinate the effectiveness of botulinumToxin (botox) in correct restrictive strabismus and eliminate the diplopia in primary position of look and reading.
Methods. we carried out a descriptive, prospective and longitudinal study to 10 patients (17 eyes) with restrictive strabismus of March to June of the 2008, using botulinum Toxin with direct method, without any electrical control, we have registered the effect and stability in strabismus and influence in the systemic disease.
Results. BotulinumToxin´injection in the affected muscle was useful to correct restrictive strabismus and offers excellent results in the elimination of the diplopia and in the patient's immediate comfort; we don't observe any influence in thyroid systemic diseases. The treatment can be to correct strabismus completely or waiting to go to surgery in a better moment. The botulinumToxin´s effectiveness could be for 4 to 6 months, but in some patients is for ever.
We concludes that the therapeutic is a very good choice to treat strabismus not only for the efficacy but also for the safe and innocuous of the direct method of application

H pylori: Treatment for the patient only or the whole family?

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Aim: To compare the effects of treatment of H pylori-infected individuals with the effects of treatment of individuals as well as all H pylori-infected family members.
Methods: H pylori-positive patients with similar demographic specifications were prospectively randomized with respect to treatment, with a triple regimen of either patients and all H pylori-positive family members living togetherl (group I) or patients only (group II). Nine months after treatment, all patients were assessed for H pylori positivity.
Results: There were 70 H pylori-positive patients in each group; patients in groups I and II lived with 175 and 190 H pylori-positive relatives, respectively. Age, sex and H pylori positivity rate were similar in both groups of relatives. Nine months after 14 d standard triple therapy, H pylori positivity was 7.1% in group I patients and 38.6% in group II patients [$P < 0.01$, OR = 8.61 95% confidence interval (CI): 2.91-22.84].
Conclusion: The present results indicate bad environmental hygienic conditions and close intra-familial relationships are important in H pylori contamination. These findings indicate all family members of H pylori-positive individuals should be assessed for H pylori positivity, particularly in developing countries where H pylori prevalence is high; they also suggest patients, their spouses and all H pylori-positive family members of H pylori-positive individuals should be treated for H pylori infection.

Anticancer Activity and Lack of Toxicity of CZ48 Administered Orally To Nude Mice Carrying Xenografts of 22 Human Tumors

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Background: Camptothecin, an alkaloid extracted from the tree *Camptotheca acuminata* and some of its derivatives were found to have potent anticancer activity against human tumors growing as xenografts in nude mice. However, these compounds were much less active against tumors of cancer patients. Further studies established that all the camptothecins were converted to their inactive form in the human blood by the opening of their lactone ring caused by the presence of human serum albumin.
Methods and Results: In order to prevent such an opening, our laboratory has developed CZ48 (Camptothecin-20(S)-propionate hydrate). We have tested it for anticancer activity administering it orally to nude mice carrying xenotransplants of 22 human cancers (4 colon, 2 lung, 5 pancreas, 5 breast, 2 melanomas, and 2 sarcomas, 1 prostate and 1 bladder) at doses of 50 mg/kg/day to 2000 mg/kg/day daily, 5 to 7 days/week. The tumors were growing subcutaneously on the back of 3 month old mice. The treatment started when tumors were measurable by caliper (200-300 mm^3). CZ48 was administered suspended in cottonseed oil.
Conclusion: Two conclusions clearly emerge from this study: 1) CZ48 is totally devoid of toxicity at the maximum doses used which were administered for more than 300 days; and 2) CZ48 is a potent anticancer drug against human cancer xenografts with a wide spectrum of action which caused complete growth inhibition in 20 out of 22 human cancers treated.

SAPPHIRE: A Structural-energetic Approach to B-cell Epitope Prediction

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Background: B-cell epitope prediction facilitates the design of antibody-binding constructs for the development of novel vaccines and immunodiagnostics. This work aimed to gain insights into the problem of B-cell epitope prediction using structural energetics.
Methods: Structural-energetic analysis was applied to peptide and protein antigens. A possible rate-limiting process of local epitope unfolding was considered for the cross-reaction of antipeptide antibody with protein antigen. Immunodominance was treated as a thermodynamically determined hierarchical steric-exclusion phenomenon. The algorithm thus developed was implemented as the computer program SAPPHIRE (Structural-energetic Analysis Program for Predicting Humoral Immune Response Epitopes), with the estimated affinity for antibody as the main criterion for epitope prediction. Predictions were rendered on the cross-reactivities of polyclonal antibodies to 38 peptides with 15 globular proteins of known structure and evaluated against published experimental data comprising 18 positive and 20 negative binding interactions.
Results: Structural-energetic parameters could not be unambiguously assigned to cysteine in view of its capacity for disulfide bond formation. The energetic contribution of histidine could not be determined in view of the uncertainty of its protonation state at physiologic pH. The binding contexts defined by the types of participating antibodies (antipeptide or antiprotein) and antigens (peptide or protein) were all fundamentally different from one another. Predictions on genuine antibody-antigen cross-reactivity could be evaluated against empirical data only with regard to interaction between antipeptide antibody and protein antigen. Maximum areas under the receiver operator characteristic curves were approximately 0.71 either with or without consideration of immunodominance.
Conclusions: 1) B-cell epitope prediction is potentially complicated by the presence of cysteine and histidine. 2) The evaluation of B-cell epitope predictions against empirical data is meaningful only if the two pertain to exactly the same types of antibody (antipeptide or antiprotein) and antigen (peptide or protein). 3) Predictions on genuine antibody-antigen cross-reactivity can be evaluated against empirical data in the case of interaction between antipeptide antibody and protein antigen.

The dynamic hypothesis of latent tuberculosis infection offers a new rational to develop future therapeutic strategies.

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Background: It has been postulated that once infected by *Mycobacterium tuberculosis*, its latent form can be retained for the whole life, dormant in old lesions. With the aid of resuscitation factors these bacilli can reactivate towards active tuberculosis. These assumptions raise at least three relevant questions to answer: (1) how can dormant bacilli remain in the lungs for the whole life? Considering the constant cellular turnover and healing of injured tissues; (2) How are the resuscitation factors provided to the dormant bacilli, while immersed in old lesions?; (3) why can a 9-month treatment with isoniazid eliminate the dormant bacilli? As isoniazid is active only against growing bacilli, this treatment should be provided for life, to avoid the reactivation of dormant bacilli.
Methods: Using experimental models of latent tuberculosis infection (LTBI) in mice, we have demonstrated that the granulomas are characterized by:
Results: (1) the drainage of nonreplicating bacilli by the foamy macrophages towards the alveolar spaces; (2) the constant formation of new foamy macrophages; (3) the presence of local immunodepression, characterized by a high apoptosis, lack of lymphocytic proliferation and anergy; (4) the reduction of the immunological response and foamy macrophages accumulation after a short-term chemotherapy, and the bacilli reactivation once is finished; (5) administration of a vaccine based on fragments of *M. tuberculosis* (RUTI) allows the control of this reactivation by inducing a polyantigenic response against secreted and structural antigens.
Conclusions: The "dynamic hypothesis" suggests that LTBI would be caused by the constant endogenous reinfection of nonreplicating bacilli. This hypothesis is the only one that may explain the efficacy of the 9-month isoniazid treatment, and supports a therapy based in the elimination of the local immunosuppression by a short-term chemotherapy, followed by a therapeutic vaccination able to generate immunity against structural antigens to detect the resting nonreplicating bacilli and to avoid its reactivation.

Molecular strategies to improve the anti-tumour activity of Zoledronic acid in prostate cancer cells

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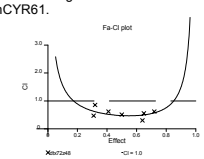
Background: Zoledronic acid (ZOL) is an aminobisphosphonate able to inhibit the prenylation of intracellular proteins through the inhibition of farnesylpirophosphate synthase. Prenylation is essential for the maintenance of the activation of components of signal transduction pathways regulating apoptosis and proliferation such as ras and ras-related proteins. ZOL has demonstrated a direct anti-tumour effect in vitro and in preclinical models and its ability in preventing skeletal related events is proven in patients with bone metastases from different origins. Clinical evidence on its direct anti-proliferative effects is emerging, but its activity is limited by the excessive accumulation in the bone. **Methods:** We describe several strategies in order to improve the anti-tumour activity of ZOL based on preclinical and biological rationales. **Results:** The first strategy is to combine ZOL with either cytotoxic drugs or other biological agents such as the farnesyl-transferase inhibitor tipifarnib focusing also on the importance of the sequence of administration of these drugs. The synergistic interaction with other agents could lower the active concentrations of ZOL allowing the achievement of anti-tumour concentrations also in extra-bone sites. The second strategy is to find new molecular targets of ZOL through the use of technological platforms such as DNA microarrays. We have analysed the gene modulation induced by ZOL in androgen-resistant prostate cancer PC3 cells with cDNA microarray platform to identify new molecular targets of ZOL in prostate cancer. The gene coding for cysteine-rich, angiogenic inducer, 61 (CYR61), often over-expressed in tumour cells, resulted highly down-regulated with a fold-change of 5.58. Therefore, we have studied the effects of different concentrations of ZOL on CYR61 protein product and we have found that CYR61 protein expression was significantly decreased after exposure to ZOL on both PC3 and DU145 cell lines. The effect of ZOL on CYR61 expression was dose and time-dependent and was due to a reduced transcriptional activity of CYR61 promoter as demonstrated by transfection with a plasmid encoding for luc-CYR61 promoter. Interestingly, other signal transduction inhibitors did not induce or induced less effect on CYR61 modulation if compared to ZOL. Moreover, ZOL reduced CYR61 expression through decreased activation of ras-raf-1-dependent pathway that was dependent from isoprenylation inhibition since they were antagonized by the addition of either farnesol or geranylgeraniol. Finally, we have investigated the role of CYR61 in the regulation of growth inhibition and invasion/motility of PC3 cells using a shRNA for CYR61 in order to down-regulate the expression of CYR61 protein. We have found that shCYR61 enhanced inhibition of proliferation induced by ZOL. Since CYR61 was reported to be involved in the resistance to taxanes we have evaluated if ZOL could sensitize PC3 cells to Docetaxel (DTX). We have found a sequence-dependent synergism induced by the combination between ZOL and DTX on PC3 cell growth inhibition and similar results were recorded after transfection of PC3 cells with shCYR61.



	Effect		
	50%	75%	
DTX → ZOL	0.475	0.623	
DTX + ZOL	0.760	1.135	CI
ZOL → DTX	0.873	0.822	

Conclusions: In conclusion, it is possible to design new molecular rationale-based therapeutic strategies in androgen-independent prostate cancer based on the use of ZOL.

Vascular disruption: an old mechanism rediscovered for targeting tumor blood supply

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Background: Vascular disrupting agents (VDAs) are cancer drugs that act through destruction of tumor neovasculature. The majority of known VDAs act through tubulin binding which leads to destabilization of microtubules in neovascular endothelial cells, increased vascular permeability, loss of perfusion and tumor necrosis. Many VDAs are currently in all phases of clinical development. The preclinical and clinical history of MPC-6827, a quinazoline-based tubulin targeting agent under development by Myriad Pharmaceuticals, is presented as a case study for discovery and development of a VDA.
Methods: MPC-6827 was identified through a drug discovery process that began with a screen for apoptosis induction in a cancer cell line and continued through biochemical, cellular and animal model characterization of activity. Subsequently, safety and efficacy of MPC-6827 was studied in two Phase I clinical trials.
Results: MPC-6827 was found to compete with colchicine for tubulin binding and to destabilize tubulin assembly using biochemical and cellular assays. MPC-6827 was also observed to potently kill tumor and endothelial cells in culture and rapidly damage tumor neovasculature, induce tumor necrosis and inhibit tumor growth in xenograft models. Furthermore, MPC-6827 did not show decreased potency in tumor cells overexpressing the ABC transporters, P-gp (MDR-1), MRP-1 and BCRP-1, which mediate multidrug resistance and maintain the blood-brain barrier. MPC-6827 demonstrated high brain availability in mice, with exposure in excess of 14-times that of plasma. A maximum-tolerated dose (MTD) of 3.3 mg/m^2 was elucidated in a Phase I clinical trial.
Conclusions: MPC-6827 is a potent cytotoxic agent that inhibits tumor growth primarily through vascular disruption. MPC-6827 is also not a substrate for ABC transporters, which likely explains the extensive brain exposure in mice. Based upon the preclinical data and upon identification of an MTD in humans, MPC-6827 has been advanced to Phase Ib/II trials in glioblastoma and melanoma.

Comparison of oral pharmacokinetics of nifedipine in different populations

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Background: Nifedipine is a calcium channel blocker that is widely used in the treatment of hypertension and angina pectoris. It has been described that this drug is importantly metabolized by CYP3A4 and differences in its pharmacokinetics have been described. In fact, it has been suggested that plasma levels are higher in Nigerians, South Asians and Mexicans when compared with Caucasians. In order to extend this information, the purpose of this study was to evaluate the oral pharmacokinetics of nifedipine in Mexicans and to compare the pharmacokinetic parameters reported in different populations by means of a meta-analysis.
Methods: Twenty male healthy volunteers were enrolled in this study that was approved by the Institutional Research and Ethics Committees. All were fit according to medical history, clinical examination and suitable laboratory tests. After an overnight fast, subjects received an oral dose of 10 mg nifedipine and blood samples were collected during 8 hours. Plasma was analysed by a validated HPLC method. Pharmacokinetic parameters were obtained by non-compartmental approach and compared by meta-analysis with those reported in different populations.
Results: Nifedipine was rapidly absorbed reaching the maximum between 30 to 60 minutes, then decayed with a half-life of about 5 hours. When pharmacokinetic parameters obtained in this study were compared with those reported in other populations by meta-analysis, it was observed that AUC reached in non-caucasian (Asian, African and Mexican) populations was almost twice the reported in Caucasians, indicating interethnic differences in the oral pharmacokinetics of this drug.

Conclusions: It is confirmed the existence of interethnic differences in the oral pharmacokinetics of nifedipine, non-caucasians reaching higher levels than Caucasians and therefore, blindy extrapolation of dosage regimens between populations is not adequate for this drug.

Cross-kingdom Vaccines : dogma and heresy

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A vaccine made up by an algal β-glucan (laminarin; β-1-3 glucan with occasional β-1-6 single glucose side chains), conjugated with diphtheria toxoid as a carrier protein component , protects against infections by different fungi and induces antibodies capable of inhibiting fungal growth .This is a sort of “cross-kingdom” vaccine because the immunizing antigen and the vaccination target belong to two different kingdoms, and this is certainly the first case in the field of human vaccines, which are generally based on the dogma “ one or more specific antigens against one disease”. Thus, it is “heretically”possible to convey in a single immunological tool the potential to protect against multiple infections, in our case all those caused by β- glucan -expressing fungi or bacteria. The generation of antibodies with the potential of directly inhibiting the growth of, or killing the fungal cells also opens an exciting perspective for both active and passive vaccination in immunocompromized subjects.
The above approach could be theoretically extended to non-fungal infections by selecting the appropriate molecular pattern shared by a given microbial group (e.g. peptidoglycan for Gram positive bacteria). Noteworthy , the molecular patterns are those microbial molecules which foster natural immunity through their binding to the pattern-recognition structures on host cells. Thus, single-component, molecular pattern-based vaccines would merge the broad target range typical of innate immunity with the highly focussed specificity of the adaptive immunity.

Heparin as anti-inflammatory and anti-metastatic drug- new potentials of an old player

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Background: Heparin is a well-known compound based on its anticoagulant activities. This molecule is a highly sulfated carbohydrate glycosaminoglycan-chain, belonging to the family of heparan sulfates. Heparan sulfates are ubiquitously expressed throughout the body, and are especially known for their roles in cell adhesion and migration, angiogenesis and wound healing. Based on the roles of heparan sulfates in physiological processes, the use of heparin as anti-inflammatory and anti-metastatic drug has gained increasing research interest.
Methods: Several studies have examined the effectivity of heparin, low molecular weight heparin and synthetic heparin mimetics in various experimental inflammatory models and tumor growth and metastasis.
Results: Heparin and its derivates have been shown to have broad anti-inflammatory properties in models of delayed type hypersensitivity, peritonitis, meningitis, allergic encephalomyelitis, airway (allergic) and cutaneous inflammation, myocarditis, as well as ischemia/reperfusion-induced inflammation in the heart, liver and kidney. The mechanism involved is likely based on the ability of heparins to block leukocyte adhesion and migration through binding of the selectin family of adhesion molecules (especially L-selectin and P-selectin), as well as chemokines and cytokines. In addition, there is increasing evidence that heparins can inhibit tumor growth and metastasis by affecting angiogenesis, cell proliferation, as well as cell-matrix interactions. We will shortly discuss the several lines of evidence.
Conclusions: Although heparin has been known and used for decades, it is becoming increasingly clear that this highly potent compound may be effective in several other clinically important settings such as (chronic) inflammation and cancer. Efforts are being made to generate heparin-mimetics with targeted specificities to enhance their potential use in clinic.

Electrochemical Behavior of Flavonoids in the Presence of Metal Ions

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Background: Flavonoids are a large group of phytochemicals ubiquitously found in many food products of vegetable origin. They have been reported to have a broad spectrum of pharmacological activity. In addition, due to the presence of hydroxyl groups in their molecular structure, flavonoids exhibit strong antioxidant properties. Previous works in this field show that their antioxidant activity is modified in the presence of metal ions by the formation of soluble complex species which change the ability to scavenge free radicals. The goal of this work was to (i) make a comparative study on the electrochemical behavior (cyclic voltammetry) of the flavonoids quercetin (qrc), rutin (rut), rhamnetin (rha) and isorhamnetin (irh) on gold electrodes, and (ii) to study the influence of the relevant metal ions Cu(II) and Fe(III) on the electrochemical oxidation of the four flavonoids.
Methods: Qrc and rut were reagent grade, purchased from commercial sources and used without further purification. Rha and irh were isolated from *B. trimera*. The isolated compounds were further characterized by HPLC-DAD analysis and HPLC co-chromatography with pure standards purchased from Indofine and Fluka.
For cyclic voltammetry experiments, a polycrystalline Au-pc disc (99.999 %, 3 mm diameter) shrouded into a Teflon body and a Pt sheet, 3.0 cm^2 geometric area, were used as working and counter electrodes, respectively. The reference was the Ag/AgCl electrode (3 M KCl, E = 0.230 V vs. SHE). The solutions were prepared by mixing the flavonoid and/or the metal ion in basic media (pH around 9) to reach 1 mM concentration in water. The ionic strength was adjusted up to 0.15 M with $NaClO_4$ assupporting electrolyte, and adjusted to the desired pH with dilute NaOH or $HClO_4$. Potential scan rates within the range 0.005 $Vs^{-1} \leq v \leq 0.05$ Vs^{-1} were employed.
Results: Evaluated free flavonoids present similar features, including many anodic contributions located in the positive potential range. Some of them can be certainly attributed to the redox behavior of the system Au-pc in the supporting electrolyte and the others arise from the electroactive moieties of the flavonoids.
Table 1. Anodic contributions ascribed to redox processes involving functional groups of the flavonoids. E(V) *vs.* SHE

	qrc	rut	rha	irh
E_{an1}	0.43	0.38	0.35	0.32
	0.56	0.45	0.53	
E_{an2}	0.87		0.68	
E_{an3}	0.97		1.13	1.07

Anodic peaks (E_{an1}) at *ca.* 0.3 V and 0.5 V could rise from the oxidation of the OH moiety from chatecol to quinone following a two one-electron steps path. Contributions (E_{an2}) at 0.68 V in rha and 0.87 V in qrc could be assigned to oxidation of the OH located in the same ring than the quinone. This process was not detected in rut, caused by to the blocking of this position with the sugar residue. In the case of irh this signal is also absent, may be due to difficulties to reach the electrode surface or the formation of an intramolecular hydrogen bond with the O of the quinone. Finally, contributions at *ca.*1 V (E_{an3}) can be attributed to the oxidation of some of the remaining OH groups. When copper is added, a new contribution (not present for the free flavonoids) is detected at *ca.* -0.2 V, assigned to the copper redox behavior itself. The strong interaction Cu(II)-flavonoid makes copper reduction less favorable. As a consequence, a poorer participation of the copper ion in the formation and propagation of the free radicals is expected. After addition of Cu, only the E_{an1} is mainly affected and appeared at less positive potentials than in the case of the free flavonoids, indicating an increased reduction power of the copper complexes in aqueous solution. When Fe(III) is added to the flavonoid containing solutions, features are quite similar to those observed for the copper complexes.
Conclusions: 1) The study of the electrochemical behavior of the flavonoids in aqueous solution is a powerful tool to understand the redox processes related to the Antioxidant Activity of these natural antioxidants. The oxidation of the catechol moiety producing a quinone can be split into two independent signals and individually analyzed. 2) Further oxidation of the remaining OH groups of the quinone can also be studied in this medium, as well as the reduction of the flavonoids. 3) Upon complexation with Cu(II) or Fe(III), the species with 1:1 stoichiometry present a similar voltammetric pattern compared to the free flavonoids. However, the oxidation of the flavonoids is thermodynamically favored, especially in the case of the Cu(II) complexes. 4) The complex species also present changes in the ability of the metal ion to be reduced, i.e., the metal reduction is less favorable in the presence of the flavonoids. The flavonoids could sequester Cu(II) and Fe(III) preventing their reduction and subsequently the formation of free radicals. 5) The influence of Cu(II) on the AA of the flavonoids, at least from a thermodynamic point of view, is more notorious than in the case of Fe(III). On the other hand, the interaction with flavonoids regarding the possibility to reduce the metal ion is also more favorable for Cu(II). **This work was accepted for publication in Inorganic Biochemistry: Research Progress, NOVA Publishers, Ed. by J.G. Hughes and A.J. Robinson.**

Treatment of Hepatic Abscess: Magic Bullets and Beyond

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Background: Constant improvements of antibiotics have had crucial influence on clinical management of pyogenic liver abscess (PLA). Advances in microbiological diagnosis as well as in interventional radiology have also contributed to better outcome, but successful treatment of PLA still remains a considerable clinical challenge.
Methods: Clinical data of a series of 76 patients with PLA were analyzed. Initially, broad-spectrum antibiotics were given, and treatment was modified according to sensitivity testing as soon as possible. When indicated, additional therapy with percutaneous puncture/drainage, endoscopic papillotomy/stenting or surgical interventions was used.
Results: The disease was confined to the right hepatic lobe in 76% of the patients and to the left lobe in 7%; in 17%, both lobes were affected. Fifty-eight patients (76%) had a single PLA and 18 patients had multiple abscesses. Etiology was biliary in 38%, hematogenous in 11%, posttraumatic in 9% and cryptogenic or attributable to rare reasons in the remaining patients. Microbiological culture was sterile in 24%, which was at least partly due to antibiotic pre-treatment. *Staphylococci*, *Streptococci* and *E.coli* were most often identified. Anaerobes were found in 15%. Factors associated with the need for surgery included empyema of the gallbladder, underlying malignancy, perforation, vascular complications (hepatic artery thrombosis) and foreign bodies (e.g., toothpick, infected ventriculo-peritoneal shunt). In patients with biliary fistulae it was important to ensure prompt bile flow (for instance, by sphincterotomy/stenting)
Conclusions: In surgical departments, we usually treat a selected group of patients with very severe forms of PLA. “Magic bullets”, adapted due to the results of microbiological testing, constitute the mainstay for treatment. However, additional therapy with interventional radiology and/or surgery was usually required in our patients and successful management of underlying diseases played a decisive role for positive outcome.

Anti-Angiogenic and Anti-Neoplastic Potential of Prostate-Specific Antigen (PSA)

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Background: In prostate cancer, disease progression and prognosis are related to angiogenesis and the degree of tumor vascularization correlates with progression and the development of metastatic disease. PSA protein present within the prostate tissue microenvironment represents the pool most critical to the pathogenesis of prostate cancer. Tissue-PSA (T-PSA) levels correlate with prognosis in prostate cancer, as well as in breast cancer. *Hypothesis*: *PSA down regulates pro-angiogenic growth factors and up-regulates anti-angiogenic growth factors.*
Methods: In vitro and in vivo studies were carried out using prostate tumor (PC-3M) cells in culture and in nude mice. We analyzed modulation of protein expression in PC-3M cells by PSA using 2D-DIGE analysis coupled with HPLC-MS/MS and SEQUEST data mining. Biological network analysis was carried out using MetaCore integrated software designed for functional analysis of experimental data. Gene expression data for several regulated proteins were confirmed by real-time, quantitative PCR. Anti-angiogenic potential of PSA was also observed using human umbilical vein endothelial cells (HUVEC) in an in vitro anti-angiogenic assay.
Results:
a. Incubation of PC-3M cells with purified PSA resulted in a significant down-regulation of expression of 147 genes including genes like VEGF, IL-8, EphA2, CYR61, Bcl2, Pim-1 oncogene, and uPA, that are associated with angiogenesis/tumor progression in different cancers and up-regulation of expression of 137 genes including IFN and IFN-related genes and peptide inhibitors of angiogenesis.
b. Forty one proteins were significantly ($p<0.05$) changed in abundance in PC-3M cells treated with PSA. Many down regulated proteins including N8 gene product long isoform, laminin receptor, Vimentin, DJ-1 and Hsp60 are known to be involved in tumor progression.
c. PSA inhibited significantly, in a dose dependent manner, the migration/chemotaxis and attachment functions required for tube formation by HUVEC.
d. PSA delivered to PC3M xenografts engrafted to nude mice resulted in inhibition of tumor growth.
Conclusions:
1. In PC-3M cells, PSA down regulates pro-angiogenic growth factors and up regulates anti-angiogenic growth factors.
2. Enzymatically active and inactive forms of PSA have anti-angiogenic activity in vitro.
3. PSA has antitumor activity against PC-3M xenografts in nude mice.

Magic Bullets Versus Shotgun Drugs in Cancer Therapy and Prevention

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Background: The pioneering ideas developed over hundred years ago by Paul Ehrlich led not only to his Nobel Prize but also to the modern day way of rational drug design. The concept of promiscuous drugs, where a single drug can target not only multiple steps in cancer progression and prevention but also infections caused by viruses and parasites, is more recent and contrasts with the shotgun approach of one target – one drug concept.
Methods: Bacterial proteins such as azurin or Laz are produced as weapons by pathogenic bacteria to prevent other intruders to invade their habitats during colonization in human tissues. Assays involving induction of apoptosis and cancer cell death as well as protein-protein interactions have shown the ability of azurin or Laz to inhibit the growth of various cancers, HIV/AIDS and malaria/toxoplasmosis-causing parasites, thus acting as a magic bullet.
Results: The ability of azurin or Laz to induce apoptosis through complex formation with tumor suppressor protein p53 or inhibit growth of cancer cells by interfering in receptor tyrosine kinase-mediated cell signaling or preventing angiogenesis has been shown. *In vivo* cancer regression by azurin has also been demonstrated. A chemically-synthesized 28-amino acid peptide derived from azurin, termed p28, is non-toxic to animals including monkeys and is under consideration by the U.S.FDA for phase I human clinical trials as an anticancer agent. Azurin and p28 can also interfere in oncogenic transformation to prevent precancerous lesion formation in mouse alveolar and ductal mammary glands exposed to a carcinogen DMBA. Many patents have been issued to cover these inventions.
Conclusions: While Paul Ehrlich emphasized human ingenuity to design new chemicals for the treatment of infections and cancer, such efforts are limited to our ability to develop validated targets. Our understanding about cancer is an evolving process and will take years for a fuller grasp. A single or few targets also allow the disease agents to change such targets to become drug resistant. Bacteria have 3 billion years of evolutionary wisdom and can design proteins as weapons that target multiple disease agents and multiple steps in the disease progression pathway to prevent rapid drug resistance. We believe such multi-targeting bacterial proteins will be our next generation drugs as envisioned by Paul Ehrlich.

Capitalizing on CYP450 polymorphism in HIV treatment optimization

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Background: The use of highly active antiretroviral therapy (HAART) is now the standard in the clinical management of HIV infection. HIV patients are normally prescribed with 2 nucleoside reverse transcriptase inhibitors (NRTI) plus one of the two other major classes of compounds – protease inhibitors (PI) or non-nucleoside reverse transcriptase inhibitors (NNRTI). As Cytochrome P450 is the major enzyme system for the metabolism of the latter two antiretrovirals, treatment response may vary from one ethnic group to another. Treatment optimization could be effected by exploiting the pharmacokinetic and pharmacoegnomic patterns.
Methods: NNRTI is commonly prescribed as a component of first-line regimen in treatment-naïve patients. The pharmacokinetics of efavirenze, an NNRTI is correlated with the CYP450 2B6 polymorphism in a Chinese population.
Results: We have studied the prevalence of G516T of CYP450 2B6 gene in healthy Hong Kong Chinese blood donors and found it to be high (TT genotype 23%; allelic frequency of T at 516 0.43) compared to Caucasian populations. Interestingly there’s also variation among different ethnic groups in Chinese population. In a followup study we recruited 68 Chinese HIV patients from a hospital in Southern China. All patients have been receiving a HAART regimen comprising of efavirenz, an NNRTI metabolized largely through CYP450 2B6. The allelic frequency of 516T in this cohort was 0.23. The TT genotype was associated with a higher level of efavirenz at 8-12 hours post-dose, as compared to GT or GG. The pharmacokinetic pattern of TT was likewise different.
Conclusions: 1) The determination of CYP genotype and allelic pattern ca be a useful adjunct in the clinical moniyoring of HIV I infection. 2) Adjustment of efavirenz dosage in accordance with CYP genotypic or allelic pattern could be a potentially useful strategy for optimizing treatment outcome and minimizing adverse effects. 3) Clinical guidelines on the use of antiretroviral ompounds may need to be refined according to the pharamcogenomic pattern of the local population.

Novel Clinically Relevant Proteasome Inhibitors and HDAC Inhibitors

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Background: Agents with specific biologic targets, that display more selective killing of leukemia cells as compared to normal lymphocytes require further study in these diseases. Here we focus on two distinct classes of new agents: proteasome inhibitors and histone deacetylase inhibitors (HDACi) and highlight a specific agent within each class as possessing unique properties with potential therapeutic benefit. NPI-0052 is a proteasome inhibitor distinct from bortezomib, which is approved by the Food and Drug Administration (FDA). PCI-24781 is an HDACi which targets a distinct class of HDAC's more specifically than does vorinostat, the only FDA approved HDACi. Here we tested these compounds in leukemia cells to determine the mechanism of cytotoxicity and to compare them to approved counterparts.
Methods: Cell lines were representative of acute myelogenous leukemia and acute lymphocytic leukemia. Cell death was assessed by measuring DNA fragmentation by propidium iodide staining and flow cytometry. Caspase activation was measured by activity assays and by western blotting. Oxidative stress was quantitated using dichlorofluorescien and dihydroethidium to measure levels of intracellular peroxides and superoxide, respectively. Proteasome activity assays were conducted using fluorogenic peptides. Histone acetylation was assessed by western blotting for histone H3 acetylation. Combination indices were based on Chou and Talalay's methods.
Results: Dose response and time course studies revealed that NPI-0052 is more potent than bortezomib and inhibits the catalytic activities of the proteasome more effectively than bortezomib. Similarly, PCI-24781 exerted unique effects, causing histone hyperacetylation at lower doses than vorinostat. Cells lacking caspase-8 did not display histone acetylation by PCI-24781. Surpisingly, NPI-0052 promoted histone acetylation which was dependent upon caspase-8 and oxidative stress. Synergy of NPI-0052 with several HDACi was stronger than seen with bortezomib.
Conclusions: 1) NPI-0052 is more potent alone and in combination with HDACi than bortezomib. This agent also acetylates histones in an oxidant and caspase-8 dependent manner. 2) PCI-24781 action was also dependent upon caspase-8, suggesting that promoting caspase-8 activity may complement the activity of both agents in leukemia.

Neomycin, Kanamycin and Pyranmycin: Synthesis, Antibacterial Activity and New Applications

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Background: Antibiotic resistance represents stringent problems for the global health. Development of new antibiotic is urgent. Utilizing synthetic methodologies (glycodiversification), we have synthesized libraries of neomycin and kanamycin classes of aminoglycosides. Novel aminoglycosides with prominent antibacterial activity against a panel of resistant bacteria including *Pseudomonas aeruginosa*, mecilline-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) have been identified. In addition, we have also discovered new applications of aminoglycosides. These include antifungal, antiviral and potential therapeutic for neural disease.
Methods: We employed two different synthetic strategies: 1) library construction of aminoglycosides using glycodiversification approach, and 2) leads construction using carbamate approach. Whole-cell based assay and molecular modeling have been employed for revealing useful structural activity relationship.
Results: From our studies on carbohydrates and aminoglycosides, we have made several important discoveries which can be divided into two aspects in chemistry and biology areas.
In chemistry area, we have invented several synthetic protocols to be used in the synthesis of diverse glycoconjugates of biological interest. In addition to the library synthesis of aminoglycosides, we have developed methods like dideoxygenation at mild condition, regioselective Staudinger reaction, and azido-migration for creating new structural scaffolds on aminoglycosides.
In biological area, one of the newly developed aminoglycosides, *pyrankacin* manifests superior activity than clinically used amikacin and gentamicin against various resistant bacteria. Two aminoglycosides exert impressive activity (low micromolar) against MRSA and VRE. In the antifungal studies, we have identified two leads with prominent activity against fungal pathogens, such as *Fusarium graminearum*. In the effort of developing potential therapeutics for the treatment of spinal muscular atrophy (SMA), we have noticed a lead with eminent activity in mouse model.
Conclusions: Through our continuous effort, we have developed new aminoglycosides with broad spectrum activity against resistant bacteria. Additionally, new activities and applications have also been discovered.

Competing Causes of Death from a Randomized Trial of Extended Adjuvant Endocrine Therapy for Breast Cancer: NCIC CTG MA.17

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Background: Older women with early-stage breast cancer experience higher rates of non-breast cancer-related death. We examined factors associated with cause-specific death in a large cohort of breast cancer patients treated with extended adjuvant endocrine therapy.
Methods: In the MA.17 trial conducted by the National Cancer Institute of Canada Clinical Trials Group, 5170 breast cancer patients (median age = 62 years; range = 32-94 years) who were disease-free after approximately 5 years of adjuvant tamoxifen treatment were randomly assigned to treatment with letrozole (2583 women) or placebo (2587 women). The median follow-up was 3.9 years (range = 0–7.0 years). We investigated the association of 11 baseline factors on the competing risks of death from breast cancer, other malignancies, and other causes. All statistical tests were two-sided likelihood ratio criterion tests.
Results: During follow-up, 256 deaths were reported (102 from breast cancer, 50 from other malignancies, 100 from other causes, and four from an unknown cause). Non-breast cancer deaths accounted for 60% of the 252 known deaths (72% for those ≥70 years and 48% for those <70 years). Two baseline factors were differentially associated with type of death: cardiovascular disease was associated with a statistically significant increased risk of death from other causes (P = .002) and osteoporosis was associated with a statistically significant increased risk of death from other malignancies (P = .05). An increased risk of breast cancer-specific death was associated with lymph node involvement (P<.001). Increased risk of death from all three causes was associated with older age (P<.001).
Conclusions: Non-breast cancer-related deaths were more common than breast cancer-related deaths in this cohort of 5-year cancer survivors, especially among older women.

The Antagonistic Role of Curcumin against Nicotine induced Genotoxicity on different Organs of Female Rats under Restricted Dietary Protein

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Background: Nicotine, a major pharmacologically active substance of tobacco for several diseases, has proven to be a potential genotoxic compound. It is absorbed through lungs with smoking and mainly metabolized in liver, yet its effect on liver injuries and other organs particularly in restricted protein diet are not clear. The aim of this study was to investigate the genotoxicity of nicotine and corresponding the protective role of curcumin on liver, ovary and uterus of female populations particularly who used tobacco but deprived of healthy diet.
Methods: The study was investigated by: 1) Measurement of total DNA concentrations and 2) Analysis of DNA damage by Comet assay in the investigated tissues of female rats maintained under altered protein diets. Two groups of female albino rats (15 animals each) were maintained in normal protein diet (18% casein) and restricted protein diet (5% casein) respectively. Each group was divided in three subgroups (5 animals each). First subgroup was served as control and second and third subgroups (experimental) was injected nicotine tartrate (2.5 mg/kg body weight/day) subcutaneously and the third subgroup was administered curcumin (80 mg/ kg body weight/day) orally. The animals were sacrificed after 21 days of treatment and the total DNA content in the specified tissues were measured. The DNA damages in the tissues were determined by Comet assay. Results were analyzed by One way Analysis of Variance, all pair wise Multiple Comparison Procedure (Holm-Sidak) and Function CORREL of Microsoft Excel.
Results: Total DNA contents of all investigated tissues were decreased more significantly (P< 0.001) by nicotine in both dietary conditions. Significant (P< 0.01) increase of total DNA content in normal diet and more significant (P<0.001) increase of that in protein restricted diet was observed due to curcumin supplementations. Curcumin more significantly (P< 0.001) reduced the DNA damage percentage of the liver tissues in protein-restricted condition.
Conclusions: 1) The degree of nicotine-induced genotoxicity increases in protein restricted condition. 2) Curcumin effectively reduces the effect of nicotine as observed in tissues. 3) The protective role of curcumin is more pronounced under protein-restricted condition.

Dose-dependent Antiherpesvirus Activity of an Ethnomedicinal Phytophores: Search for Magic Bullet

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Background: Ophiorrhiza nicobarica Balkr., a wild herb popularly used as an antiinfective medicament by the Shompen and Nicobarese tribes of Grate Nicobar Islands, India, was investigated for its anti-herpesvirus and antibacterial activities.
Methods: The whole herb was extracted in water and ethanol, and fractioned in n-butanol for the isolation of bioactive compounds using TLC, HPLC, HPTLC and NMR. The in vitro and in vivo toxicity of the extract were determined in Vero cell and in mouse model. The antiviral activity and its mechanism was tested by Cytopathic effect (CPE), Plaque reduction assay, Yield reduction assay, and Dose response curve using the Herpes Simplex Virus type 1 (HSV-1) and HSV type 2 (HSV-2). The EC50 (50% protection against virus induced cytopathic effect) and Selective Index (SI; ratio of 50% cellular cytotoxicity to EC50) was determined. The antibacterial activity was tested by disc diffusion and agar dilution methods on 150 bacterial isolates of human origin, and the mechanism was tested by biochemical and transport studies.
Results: The extract was non-toxic up to 3.6 mg ml-1, while at 300 mg ml-1 it completely inhibits plaque formation in HSV-1 and HSV-2. The antibacterial activity was noticed at 128-2000 µg ml-1 against 25 Gram negative and 53 Gram positive isolates. These data are highly significant (p<0.05) compared to the control. The bioactive part of the extract contains ursolic acid (triterpene), quercetin (flavonoid), ?-sitosterol and harmine (?-carboline indole alkaloid). The isolated ursolic acid and quercetin in combination (100?g ml-1 each) showed highly significant (P>0.001) anti-HSV activity (EC50 = 37.2 for HSV-1 and 45.1 for HSV-2), while harmine showed moderate anti-HSV activity at 300?gml-1 (EC50 = 74.4 for HSV-1 and 82.2 for HSV-2). Interestingly the eclipse phase initiation of HSV-2 was delayed at 100 mg ml-1 of ursolic acid. The yield reduction inhibition was 85% with ursolic acid, 68% with harmine and 51% with quercetin respectively for HSV-1. Moreover, the ursolic acid in combination with acyclovir showed the highest activity (EC50 20.1 and 32.2, SI was 87.5 and 90.4 respectively). The results revealed that the early stage of multiplication was blocked by quercetin while ursolic acid and harmine block the late stage of multiplication. While membrane destabilization in susceptible bacteria is noticed with ursolic acid (50-100?g ml-1).
Conclusion: The O. nicobarica extract have good anti-HSV activity, along with moderate antibacterial activity possibly due to its ursolic acid, quercetin and or harmine content. Studies showed that the extract is acting on early and late stage of HSV multiplication and have SI value grater than 20. These results suggest that this herb have the potential in the management of HSV infections, particularly in primary health care.

Blocking Effect of an Immuno-Suppressive Agent, Cynarin, on CD28 of T-Cell Receptor Found by a Novel Pharmaceutical Method

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Background: Resting T-cells are stimulated to initiate immune activity in response to specific external stimuli. The "signal 2" pathway is in part initiated by a binding interaction between CD28 (T-cell receptor) and CD80 (B-cell receptor). Recent efforts to find new immuno-suppressive drugs have focused on cell membrane receptor's antibodies. However, using antibodies as drugs has many drawbacks. Also, unlike small molecules, which mimic metabolites in the body, the large antibodies might induce other immune system problems and therefore reduce their therapeutic utility.
Methods: In this study, cynarin was purified from *Echinacea purpurea* extracts on a silica gel open column with serial elution buffers. Based on HPLC analysis, the active fraction including cynarin was collected at a retention time of 32.612 min. The UV absorption peak shifted from 338 nm (crude extract) to 330 nm (cynarin pure fraction). The cytotoxicity of cynarin treatment of T-cells was measured by MTT colorimetric assay. Molecular modeling and docking among cynarin and CD28/CD80 were done by PRODRG2 based on AutoDockTools.
Results: Cynarin, a potential immunosuppressant that blocks the interaction between the CD28 of T-cell receptor and CD80 of antigen presenting cells, was found in *Echinacea purpurea* extract by a new pharmaceutical screening method: After Flowing Through Immobilized Receptor, (AFTIR; G.-C. Dong *et al*., *J. Med. Chem.*, 2006, 249: 1845-1854). This *Echinacea* component is a small molecule that is able to specifically block "signal 2" of T-cell activation. In this study, we further confirm that cynarin effectively blocked the binding between CD80 of B-cells and CD28 of T-cells, and provide details of its mechanism of action.
Conclusions: The results above confirm both that AFTIR is a promising method for screening selective active compounds from herbal medicine and that cynarin has great potential as an immuno-suppressive agent.

Synergistic inhibition of taxol- reisistance primary ovarian cancer cells by oridonin and wogonin

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Abstract: In researching the molecular principle of Chinese herbal medicine for cancer therapy, we use the composition of the original US phyto product "PC SPES" as a study model. Now no longer available, PC SPES was reported by several research groups to be active in suppressing hormone refractory prostate cancer. Among the isolated phytoactive chemicals, baicalein, oridonin isoliquiritigenin and wogonin were found to individually or combinatorially inhibit the ovarian cancer cell lines sensitive (A2780) or resistant (PTX10) to taxol. The activity was confirmed by MTS cell viability assay, SRB antiproliferation assay and colony formation assay. Although the four agents share some common molecular targets including the inactivation and down regulation of transcription factors NF-kB and androgen receptor, oridonin seems to display the most potent activity in modulating cancer cell apotosis and stabilization of p53 protein. To fully understand the action of botanical medicine, it is essential to investigate the combinatory effect of active agents. Our investigation has led to the discovery that differential synergism is dependant on the cancer cell type. While the strongest synergistic inhibition was observed in prostate cancer cell line DU-145 (isolated from hormone therapy refractory patients and androgen receptor insensitive) induced by the combination of oridonin and baicalein, the combined pair of oridonin and wogonin exhibited the most potent antiproliferative activity in ovarian cancer cells A2780 and PTX10. Since taxol is the last treatment option in ovarian cancer therapy, we further tested our finding on the primary ovarian cancer cells directly isolated from the ascitic fluid of patients. Preliminary results reveal that the individual and combined agents markedly suppress the proliferation of primary cancer cells. In contrast, taxol failed to inhibit both primary cancer cell cultures up to a concentration of 100 nM. In view of the different chemical structure and biological activity of terpenoids and flavonoids, we are interested to understand the scientific rationale of the combination. On this ground, we presently are studying the underlying molecular mechanism and the systems biology network affected by phytochemicals. This report discusses our initial findings. We thank Dr. Marianne Poruchynsky of NIH (Rockville, Maryland) for the gift of PTX10 cell line.

From Mono- to Dimeric-IRFs: The Heart of the Matter in Activation of the Interferon Regulatory Factors

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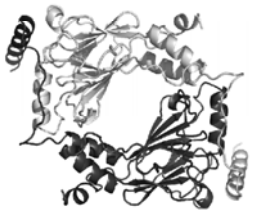
Background: Members of the Interferon Regulatory Factor (IRF) family of proteins play important roles in development of the immune system, host defense, inflammation, apoptosis and tumorigenesis. Activation of these proteins in the cytoplasm is triggered by phosphorylation of Ser/Thr residues in a C-terminal autoinhibitory region. Phosphorylation stimulates dimerization, transport into the nucleus and assembly with the coactivator ***cAMP response element-binding binding protein*** (CBP)/p300 to activate transcription of type I interferons and other target genes. However, it is unknown how the phosphorylation of IRF proteins activates dimerization and why dimerization favors binding of CBP/p300.
Methods: To that end, x-ray crystallography, size-exclusion chromatography, isothermal titration calorimetry, and mutational studies are used in this study.
Results: We present here that the 2.0Å resolution crystal structure of a dimeric form of the IRF-5 transactivation domain (residues 215-477) in which Ser 440 has been mutated to the phosphomimetic Asp. The structure reveals a striking mechanism of dimerization in which the C-terminal autoinhibitory domain attains a highly extended conformation permitting extensive contacts to a second subunit. Based on comparison with crystal structures of IRF-3, these results provide a structural basis for the coupling between dimerization and CBP binding in IRF family members, in which the C-terminal autoinhibitory domain plays a dual role. In the unphosphorylated form, the C-terminal autoinhibitory domain binds to and masks the hydrophobic CBP/p300 binding surface.
Conclusion: Phosphorylation stimulates the unfolding of the C-terminal autoinhibitory domain which then forms extensive contacts with a second IRF-5 subunit, leaving a hydrophobic surface free for binding CBP/p300.



All abstracts are listed in alphabetical order of the presenting author.

iTRAQ-coupled 2D LC-MS/MS Analysis of Proteomics Profile in Cells Incubated with *S*- or *R*-enantiomers of Chiral Drugs: from Metabolic Pathways to Biomarker Discovery

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Background: Protein profile in cells incubated with individual enantiomers of chiral drugs is important to understand their differential mechanisms of action. Using propranolol, a β-blocker used for controling hypertension and myocardial infarction, the main aims are: 1) To establish cellular protein profile by LC-MS/MS apparoach. 2) To identify specific proteins secreted from cells incubated with propranolol.
Methods: The vascular smooth muscle A7r5 cells were cultured and incubated with the *S*- or *R*-propranolol at a concentration which showed no significant effect on the cell viability. A 4-plex multiplex strategy was used to simultaneously detect and quantitate differences in intracellular as well as extracellular proteins in untreated vascular smooth muscle cells and those incubated with *S*- or *R*-propranolol. The analysis was performed on an Agilent 1200 nanoflow LC system interfaced with a QSTAR XL mass spectrometer (Applied Biosystems). Relative abundance quantitation and protein identification were performed using ProteinPilot™ Software 2.0 (Applied Biosystems).
Results: A total of 40 and 13 unique proteins were identified in three independent experiments in cells and culture medium of cells incubated with *S*- or *R*-propranolol, respectively. For intracellular proteins, the higher level of enzymes involved in cellular anabolism and antioxidant activity in cells incubated with the S-propranolol was supported by Real-Time PCR and Western blot analyses. The increase in the anabolic activity was also supported by the higher intracellular concentration of the metabolic cofactor NAD^+. For extracellular protein, only T-kininogen was found to be significantly increased in cells incubated with *S*-propranolol by Western blot analysis.
Conclusions: 1) Metabolic effect associated with propranolol treatment was revealed. The relevant metabolic enzymes may be useful targets for future pharmaceutical interventions to reduce clinical side effects following propranolol treatment. 2) The higher level of secreted T-kininogen from cells incubated with *S*-propranolol may provide a link of this vasoactive peptide to treatment of cardiovascular disease. 3) Our approach may be a platform for drug-target cells analysis and biomarker discovery.

New targets for new drug discovery opportunities.

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Today most pharmaceutical companies use similar strategies to target the same proteins. As a consequence there is a very high competition between the different pharma, a reduction of the potential markets, a lower innovation and a limited freedom to operate. Overall the productivity is decreasing. The identification of new drug targets is then very important for the future of the pharmaceutical industry.
During this presentation we shall examine the ATPases as a new family of drug targets. From a brief structural analysis of the ATP-binding site of these enzymes, we shall see that some these enzymes are very good drug targets for a strategy aiming for the identification of ATP competitive inhibitors.
Finally, we will show that it is possible to identify low molecular weight inhibitors of these enzymes and to develop them up to the clinic.

Association between Alpha-2a-Adrenergic Receptor Gene and Methylphenidate Response in Korean Children and Adolescents with ADHD

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Background: Methylphenidate (MPH), known to be effective for the attention deficit problems, blocks norepinephrine transporters and low oral doses of MPH have more effect on norepinephrine than on dopamine in subcortical areas. Alpha-2a-adrenergic receptor (ADRA2A) is a key component of the noradrenergic system. The aim of this study was to evaluate the association between the ADRA2A polymorphism and the clinical improvement of symptoms with MPH treatment in Korean subjects with ADHD.
Methods: This study included 114 ADHD children (mean age= 9.08±1.94 years) who were recruited from the child psychiatric clinic at university hospital in South Korea. The subjects who had an greater than or equal to 50% compared with the baseline ADHD rating scale (ARS) scores and who had 1 or 2 point of Clinical Global Impression–improvement (CGI-I) score after 8 weeks of treatment were considered as the 'good response' group. After performing genotyping for ADRA2A, we examined the correlation the ADRA2A polymorphism with MPH response and also compared the change of total ARS scores between genotypes at ADRA2A.
Results: e found that while 76.9% of the subjects with G/G genotype showed a good response, 46.0% and 41.7% of the subjects with C/G and C/C genotype showed a good response to MPH treatment according to ARS assessed by parent (Pearson χ^2value=11.929, df=2, p=.003). We also found a significant difference of the change at total ARS scores between the subjects with and without G/G. (t=2.21, df=1, p=.029). In terms of treatment response according to the CGI-I, significant correlation was found between genotypes at ADRA2A (Pearson χ^2value=7.250, df=2, p= .027).
Conclusions: Our findings provide evidence of an association between the ADRA2A genotype and response to MPH treatment assessed by both parents and clinician in ADHD subjects.

From Gene Expression Profile to Identification of Molecular Target Granulin-Epithelin Precursor for Liver Cancer

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Background: Primary liver cancer, hepatocellular carcinoma (HCC), is the third cancer killer worldwide. The majority of HCC patients have no effective therapeutic option. Aims: 1) To identify novel therapeutic target from the HCC microarray genome-wide expression profiles. 2) To develop neutralizing monoclonal antibody (mAb) against the therapeutic target and examine its functional effect.
Methods: The expression profiles of more than 200 HCCs and adjacent liver tissues were examined. Genes that demonstrated differential overexpression in significant portions of tumors, revealed clinically relevant biological functions, and preferably a secretory factor (diagnostic therapeutic concern) were short listed for further evaluation. The granulin-epithelin precursor (GEP, also named progranulin, acrogranin, or PC-derived growth factor) was identified as potential therapeutic target. The GEP expression on mRNA level was subsequently validated by real-time quantitative RT-PCR, on protein level by western blot and immunohistochemistry. The GEP mAb was raised against the peptide designed at the carboxyl-terminal of GEP. Neutralization effect was examined on hepatoma cells and normal liver cells *in vitro* and *in vivo*.
Results: The GEP overexpression was demonstrated in more than 70% of HCC tissues. GEP controls HCC cells proliferation, invasion and tumorigenicity. These *in vitro* and *in vivo* data corroborated the clinical findings that GEP overexpression is associated with aggressive cancer features including large tumors, venous infiltration (micrometastasis) and early recurrence after curative surgery. The GEP mAb inhibited the growth of human hepatoma cells Hep3B and HepG2, but revealed no significant effect on normal liver cells MIHA. In the nude mice model transplanted with human HCC cells Hep3B, GEP mAb decreased the serum GEP level and inhibited the growth of established tumors in a dose-dependent manner. The GEP mAb reduced tumor cell proliferation via the p44/42 MAPK and Akt pathways, and reduced tumor angiogenesis with reduced microvessel density and vascular endothelial growth factor (VEGF) level.
Conclusions: 1) GEP is a novel therapeutic target for liver cancer treatment. 2) GEP mAb inhibit tumor growth in a dosage dependent manner with anti-proliferative and anti-angiogenic functions.

Acknowledgement: The research work was supported in part by the Sun C.Y. Research Foundation for Hepatobiliary and Pancreatic Surgery, the Seed Funding Program of the University of Hong Kong, and the Hong Kong Research Grants Council (HKU 7392/03M, HKU 7560/06M, HKU 1/06C).

Application of Superparamagnetic Nanoparticles in Purification of Plasmid DNA and Recombinant Protein from Bacterial Cells

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Background: The purification of plasmid DNA or recombinant protein is fundamental to life science research, but some isolation methods can be physically and chemically damaging. Magnetic separation offers a gentle alternative. Targets are captured on magnetic particles coated with a target-specific surface, and separated from the sample using a magnetic field.
Methods: Nanosized superparamagnetic nanoparticles (Fe_3O_4) were prepared by chemical coprecipitation method using Fe^{2+}, Fe^{3+} salt, and ammonium hydroxide under a nitrogen atmosphere. A quick and reliable method for the isolation and purification of transfection-grade plasmid DNA has been developed, using PEI-modified magnetic nanobeads as a solid-phase adsorbent. We demonstrated a useful plasmid, pRSETB-EGFP, encoding the green fluorescent protein with T7 promoter, was amplified in DE3 strain of *E. coli*. A new immobilized metal ion affinity (IMA) adsorbent containing superparamagnetic nanoparticles and coated with hydrophilic resins are also proposed to improve the purification of His-tagged proteins.The GMA-IDA-coated magnetic Fe_3O_4 were employed for the direct extraction of recombinant protein, EGFP-$(His)_6$, from E. coli lysates as a model system.
Results: Up to approximately 819 μg of high-purity (A_{260}/A_{280} ratio=1.86) plasmid DNA was isolated from 100 ml of overnight bacterial culture. The eluted plasmid DNA was used directly for restriction enzyme digestion, bacterial cell transformation and animal cell transfection applications with success.The Cu^{2+}-charged GMA-IDA-coated adsorbent had the highest yield and purification factor at 70.4% and 12.3, respectively.
Conclusions: 1) PEI-modified magnetic nanobeads deliver significant time-savings, overall higher yields and better transfection efficiencies compared to anion-exchange and other methods. 2) GMA-IDA-coated magnetic adsorbent could be used as a suitable adsorbent for recombinant His-tagged protein from aqueous solution. Results proved that this new protein purification adsorbent provides a fast and efficient method for purifying His-tagged proteins with high yield and low background.

PATZ1 gene has a critical role in the spermatogenesis and testicular tumors

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Background: PATZ1, also named MAZR or ZSG, is a recently discovered ubiquitously expressed transcriptional regulatory factor gene whose product binds to the RING finger protein RNF4 that in turn associates with a variety of transcription regulators. Due to the presence of the POZ domain, PATZ1 acts as a transcriptional repressor affecting the basal activity of different promoters. Recently, it has been shown that PATZ1 is an androgen receptor (AR) coregulator that acts by modulating the effect of the AR coactivator RNF4.
Methods: To gain insights into its biological role, we generated mice lacking the *PATZ1* gene (*PATZ1*$^{-/-}$)and utilized different technical approaches (Northern blot, Western blot and immunohistochemistry analyses) to characterize the expression *PATZ1* gene.
Results: Male *PATZ1*$^{-/-}$ mice were unfertile, suggesting a crucial role of this gene in spermatogenesis. Consistently, most of adult testes from these mice showed only spermatogonia and few spermatocytes, associated with increased apoptosis, and complete absence of spermatids and spermatozoa, with the subsequent loss of tubular structure. The analysis of PATZ1 expression, by Northern blot, Western blot and immunohistochemistry, revealed its presence in Sertoli cells and, among the germ cells, exclusively in the spermatogonia. Since *PATZ1* has been indicated as a potential tumor suppressor gene, we also looked at its expression in tumors deriving from testicular germ cells (TGCTs, carcinoma *in situ*, seminoma, teratoma, and embryonal carcinoma). Although expression of PATZ1 protein was increased in these tumors, it was delocalized in the cytoplasm, suggesting an impaired function.
Conclusions: These results indicate that PATZ1 plays a crucial role in normal male gametogenesis and that its up-regulation and mis-localization could be associated to the development of TGCTs.

Pathological Studies on Thymic Lymphoma in Medaka Fish

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Background: Five orange color strain of Japanese medaka (Oryzias latipes) fish, age from 8 to 24 months old, were diagnosed with thymic lymphoma. Grossly, the tumors protruded unilaterally out of the side of the head from the thymus.
Methods: Specimens were anesthetized with MS-222. Peripheral blood smears and tumor cells imprints were stained by Giemsa–Wright stain. The bodies of victims were fixed in Bouin's fluid ready for histopathological study. Obtained tumor cells and tissues were fixed by 2.5% glutaraldehyde in 0.1 M cacodylate buffer for SEM and TEM studies.
Results: Metastatic lesions had infiltrated via both direct extension and vascular system in all fish by histopathological examination. The neoplastic mononuclear cells with transverse splitting were observed in blood smears. Mitotic neoplastic cells commonly appeared two or three nuclei in tumor tissue imprint. No virus particles was found after the investigation by TEM examination. The results obtained from SEM studies, the thymic neoplastic cells obtained from victims were identified to neoplastic lymphocytoid and lymphoblastoid. The surface of lymphoblastoid was smooth with some lamellate and pits. In addition, lymphocytoid cells had microvilli in appearence. The results obtained from TEM studies, lymphoblastoid possessed nuclear pockets and swollen mitochodria. Besides, lymphocytoid possessed few cytoplasm and vesicles inside the cells.
Conclusions: The thymic lymphoma presented in medaka at least origined from two different kinds of cells. The lymphoblast lymphoma/leukemia type showed aberrance in nuclear and edema in mitochondria. The lymphocytic lymphoma/leukemia showed aberrance in few cytoplasm, and nuclear cleft. The victims must suffer severely from anemia and neoplastic cell proliferation.

Mifepristone Acts as Progesterone Antagonist of Non-genomic Responses but Agonist of Immunosuppression in Human T Cells

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Background: Progesterone is an endogenous immunomodulator to suppress T cell activation during pregnancy. The stimulation of membrane progesterone receptors might be the cause for rapid non-genomic responses in elevating intracellular calcium ($[Ca^{2+}]_i$) and decreasing intracellular pH (pH_i) in human T cells. Mifepristone (RU486) exhibits mixed agonist/antagonist effects of progesterone on immune cells. It is necessary to explore whether these complicated effects come from RU486 being antagonist of rapid non-genomic response by progesterone.
Methods: The responses in pH_i and $[Ca^{2+}]_i$ changes were measured using the fluorescent dyes, BCECF and fura-2, respectively, in T cells. Proliferation was determined by [^{3}H]-thymidine incorporation into phytohemagglutinin (PHA)-stimulated T cells.
Results: Equimolar amounts of the progesterone antagonist RU486 blocked the progesterone-mediated non-genomic responses on $[Ca^{2+}]_i$ increases and pH_i decreases. RU486 did not block the inhibitory effects of progesterone on PHA-stimulated T cell proliferation. Rather, RU486 was inhibitory. This inhibitory effects on proliferation caused by progesterone and RU486 were additive.
Conclusions: These results demonstrate that RU486 has dose-dependent mixed progesterone antagonist/agonist effects in T cells. RU486 is antagonistic to progesterone-stimulated non-genomic responses, but agonistic and synergistic with progesterone to suppress PHA-stimulated T cell proliferation. Our findings suggest a new light on the clinical application of RU486.

Dual mechanistic anorexigenic and anti-adipogenic therapeutic for the treatment of obesity

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Background: The sharp rise in obesity in the last decade is one of the most serious public health risks worldwide. Currently approved therapies for obesity exhibit modest efficacy and limiting side effects. We show here the identification of a novel pharmacological agent, ECP00068, for the treatment of obesity.
Methods: The morbidly obese leptin-deficient, *ob/ob*, and the leptin receptor-deficient *db/db* mice, as well as the glucose intolerant/type II diabetes proned *Ceacam*$^{-/-}$, and the diet-induced obese (DIO) mice, were treated with ECP00068. Effects of ECP00068 on the differentiation of preadipocytes into adipocytes and on differentiated adipocytes were examined. Mechanisms of action of ECP00068 was investigated by whole genome DNA microarray during the differentiation of preadipocytes into adipocytes.
Results: We showed that ECP00068 causes appetite suppression that results in up to 50% reduction in food intake, decrease in visceral and subcutaneous adipose tissues, and weight loss in *ob/ob* and *db/db*, *Ceacam*$^{-/-}$, and DIO mice. ECP00068 inhibits the proliferation and differentiation of preadipocytes, and causes either dedifferentiation or delipidation of adipocytes. Gene expression profiling showed that inhibition of differentiation by ECP00068 was accompanied by the transcriptional inhibition of a large cluster of fat regulatory genes with functional equivalence in *C. elegans*. Additionally, ECP00068 increases the expression of a transcriptional repressor, zinc finger protein 68 (Zfp68), which inhibits the CCAAT/enhancer binding proteins (C/EBPs) and the peroxisome proliferator-activated receptor γ (PPARγ).
Conclusions: 1) The unique dual anorexigenic and anti-adipogenic pharmacological profile of ECP00068 suggests a first-in-class anti-obesity drug. 2) Drug-induced tolerance resulting from chronic pharmacotherapy for obesity can be overcome by a cyclical drug holiday treatment strategy. 3) Zfp68 is a repressor of C/EBPα and β, and PPARγ, master regulators of adipogenesis.

A potential molecular link for metabolic stress and carcinogenesis: AMP-activated protein kinase

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Background: AMP-activated protein kinase (AMPK), which is a serine/theronine protein kinase, is found to be a key regulator in glucose and lipid metabolism in response to cellular stress and hormones such as leptin and adiponectin. Metabolic stresses, like heat shock, hypoxia or ischemia, and glucose deprivation, have been demonstrated to activate AMPK activity. Activation of AMPK aims to switch off pathways that consume ATP, like inhibition of carbohydrates and lipid synthesis; while switch on pathways that generate ATP, like fatty acid oxidation, glucose transport and glycolysis. Recently, AMPK has been shown to be the molecular target of a wildly used anti-diabetic drug, metformin. Recent publications suggest that AMPK activation results in suppressing cell proliferation. AMPK lies upstream and downstream of two tumor suppressors, TSC2 and LKB1, respectively, indicating that AMPK may also involve in carcinogenesis. Liver cancer (hepatocellular carcinoma, HCC) is one of the most common cancers worldwide. However, the molecular mechanism underlying the development of HCC is still unclear. Here, we examined if AMPK is related to HCC formation.

Methods and Results:
Using real-time quantitative PCR, we observed that AMPKα2 was significantly underexpressed in HCCs (47.6%) as compared to its corresponding nontumorous liver samples. To further confirm the effect of AMPK on hepatocarcinogenesis, we established stable HCC clones expressing short-hairpin RNA (shRNA) that can specifically knockdown the endogenous AMPKα2 and assay for the proliferation rate as well as anchorage-independent growth in soft agar. The results showed that the stable AMPKα2 knockdown clones proliferated much faster and formed more colonies than the vector control. Since p53, an important metabolic checkpoint protein, was recently reported to be regulated by AMPK, we query whether AMPK-regulated HCC cell growth is p53-dependent. Using *in vitro* kinase assay, we showed that AMPKα2 catalytic domain can directly phosphorylate p53. Interestingly, we found that AMPK phosphorylated p53 at a novel site T150, which affect the stability of p53 protein.

Conclusions: our results suggest that AMPK may mediate its tumor suppression function through regulation of p53 in HCC.

An Entry to Clavams from Chiral Vinyl Ethers and Chlorosulfonyl Isocyanate

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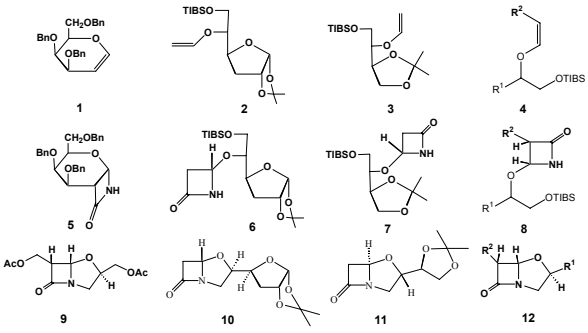
[2+2]Cycloaddition between chiral vinyl ethers **1-4** and chlorosulfonyl isocyanate leading to corresponding β-lactams **5-8** was investigated. Reactions proceeded with excellent diastereoselectivity. The special attention was focused on the problem of stereocontrol in the formation of a desired configuration of the C-4 carbon atom of the azetidinone ring. Adducts **5-8** were transformed into clavams **9-12**.



R^1=Ph, Furyl; R^2=H, Me; TIBS = 2,4,6-triisopropylobenzenosulfonyl

The adduct **5** was subjected to the sequence of reactions, in which glycolic cleavage and intramolecular alkylation of the nitrogen played crucial roles. Adducts **6-8** were transformed into clavams **10-12** via intramolecular alkylation of the nitrogen atom.

The antibacterial and antifungal properties of all clavams obtained were investigated to show, however, only in few cases interesting activity.

Bulletprooves to Abacavir-related Hypersensitivity Reaction in HIV-1-infected Population

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Background: Current antiretroviral (ARV) therapy are effective, allowing HIV-positive individuals to live longer, but at the same time they have resulted in many drug-related complications. One of it, is hypersensitivity reaction (HSR) to abacavir (ABC)treatment. Pharmacogenetics associations can influence on HIV-1 treatment. Few recent studies have shown that patients who were positive for HLA B*5701 alleles have a high risk allergic reaction to abacavir. The individual loci of alleles haplotype has been combined to susceptibility to ABC-HSR. Prevalence of HLA B*5701 among Caucasian are 5-7%.
Methods: This is retrospective analysis of clinically diagnosed ABC-HSR among 217 treated with abacavir-containing ARV regimen HIV-1 infected individuals, hospitalized between 2003-2008 in the Hospital for Infection Diseases in Warsaw. The aim of analysis was attempt to determine of *B57 alleles carriage in 12 patients (M-9, F-3, av. age 39), who developed ABC-HSR incidence. Multiple symptoms were observed in this cases, defined as abacavir-related allergic reaction, according to HSR characteristic signs and time onset between 1-6 weeks after abacavir initiation. The HLA test was performed in all 12 patients defined as ABC-HSR, using of method Sequens Based Typing [Atria Genetics].
Results: During recent 5 years, abacavir-containing antiretroviral therapy received 217 hospitalized patients. In 12 (5,5%) out of this group have clinically confirmed ABC-HSR and discontinued ABC treatment in consequence. Simply symptoms, such as skin and gastrointestinal reaction, were observed in 4 (33%) individuals. In 2 other (17%) constitutional and severe respiratory symptoms were reported. Among 6 other (50%), 3 or more HSR signs including fever, paresthesis, hypotension, tachycardia, liver parameters elevation, were observed. The onset of HSR occurred between 6 day and 6 week afterwards. No death registered. The HLA B*5701 test performed in subsequence HSR, using the sequence typing method. Only 1 patient was HLA B*5701 positive. It concernd 36y men with AIDS-C3 category, who received 5-th ARV-drug regimen due to resistance and intolerance previous antiretroviral agents.
Conclusions: 1. HLA B*5701 screening in HIV-1-infected individuals has the predictive value to ABC-HSR risk. As a result, cases of abacavir-related hypersensitivity reaction incorrectly diagnosed clinically, as well as abacavir unnecessarily discontinuation, can be reduced in clinical practice.

The Therapeutic "Cure" of Xenograft Tumors by the Third Generation Epothilone: Iso-oxazole-Fludelone

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17-Iso-oxazole-26-F_3-9,10-dehydro-12,13-desoxy-epothilone B (Iso-Flu or KOS-1803) is designed by diverted total synthesis based on pharmacological property. In vitro, it has an IC_{50} of 0.27nM against CCRF-CEM cells, which is 4-x more potent than paclitaxel (taxol). It is 638-x and 637-x more potent than taxol against drug-resistant CCRF-CEM/taxol and CCRF-CEM/vinblastine cells, respectively. It showed microtubule-stabilization activity similar to taxol. When compared with dEpoB and dehydelone, Iso-Flu is metabolically stable in mouse plasma when delivered as either a 6 hr-i.v. infusion or oral administration; has improved water solubility; has increased bioavailability and tissue penetration for prolonged tumor tissue drug retention; and demonstrates favorable pharmacokinetic and pharmacodynamic properties. The critical-optimal conditions for therapy and low toxicity are 6 hr-i.v. infusion at 25-30mg/kg every 14 days, 3x doses. In human xenograft tumors: for CCRF-CEM/taxol at optimal conditions, Iso-Flu led to 4/4 complete remission (CR) without any relapses for over 3 months, whereas taxol had no therapeutic effect, and cyclophosphamide (CTX) suppressed tumor without a CR. For CCRF-CEM xenograft, Iso-Flu, taxol and CTX all achieved CR, but 2/4 of CTX treated relapsed in one month. For the pancreatic Bx-CP-3 xenograft, Iso-Flu (25mg/kg, Q12Dx3, i.v. infusion) led to suppression and shrinkage but no CR, whereas taxol (20mg/kg, Q2Dx8, i.v.) and gemcitabine (40mg/kg, Q2Dx10, i.v.) suppressed tumor growth only 90% and 60%, respectively. For hepatoma Hep, Iso-Flu, (30mg/kg, Q16x2, i.v. infusion) led to growth suppression and shrinkage but no CR, whereas taxol (25mg/kg, Q2Dx4, i.v.) and CTX (50mg/kg, Q2Dx3, i.v.) and 5-fluorouracil (40mg/kg, QDx5, i.v.), led to only 15%, 55%, and 30% growth suppression, respectively. The superior data are in addition to early results for Iso-Flu against mammary MX-1 (i.v. infusion or oral), neuroblastoma SK-NAS, lung A549 and A549/taxol, ovarian SK-OV-3, mammary MCF-7/Adr xenografts over other chemotherapeutic agents [Chou, et al. Proc. AACR 47: 115, 2006; 48: 342, 2007, and Chou, et al. PNAS (in press)]. Thus, so far, we have not been able to find any other cancer therapeutic agent that is even approximate to Iso-Flu in overall therapeutic efficacy in xenograft experimental systems.

Authors' disclosure statement:

The intellectual property rights for epothilones at Memorial Sloan-Kettering Cancer Center have been licensed to Kosan/Bristol-Myers Squibb Pharmaceuticals.

Deferoxamine and Defersirox as magic bullets against iron overload

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Patients with anemias such as thalassemia, sickle cell disease, refractory anemias and myelodysplastic syndromes, aplastic anemia, requiring multiple blood transfusions for their survival develop hemosiderosis and/or hemochromatosis. The excess iron gets deposited in the liver, heart and endocrine organs leading to multiple organ failure. It is a major cause of morbidity and mortality in these patients. Iron overload is manifest as elevated liver iron concentration (LIC) and elevated serum ferritin levels. An increased risk of iron-induced cardiac disease has been observed when LIC values exceed 15 mg of iron per gram of dry weight (15 mg/Fe/g/dw), and serum ferritin values above 2500µg/L. Liver biopsy has been the "gold standard" for iron balance studies, but the technique is invasive procedure, expensive, and subject to variability within and between research subjects. Liver iron concentration (LIC) has been measured non-invasively by biomagnetic susceptometry using a low critical temperature (low-TC) superconducting quantum interference device (SQUID) biomagnetometer, which allows the measurement of the paramagnetic susceptibility of the iron stored in the liver as hemosiderin and ferritin. The results of biomagnetic susceptometry measurements of hepatic non-heme iron have been reported to be strongly correlated with those obtained by conventional analysis of liver biopsy. Another technique used is T2* imaging of heart. Low T2* values {<8 ms compared with (>20ms)} are related to risk of heart failure and death in iron-overloaded thalassemic patients.
Bullets as Iron Chelators: Deferoxamine: It was the first chelator developed and has been used extensively in multiple disorders needing iron chelation. It is a hexadentate chelator, it binds iron tightly, and the iron-DFO complex is excreted in both urine and stool. More than 40 years of clinical experience with deferoxamine (Desferal, DFO) in iron-overloaded patients it reduces iron-related complications and thereby improves quality of life and overall survival, when administered in adequate doses over long periods. The standard regimen to remove excess iron is by subcutaneous (sc) infusions of DFO over 8-12 hours, on 5 to 7 days each week because of short plasma half-life. The DFO-iron chelate is charged and does not readily enter and leave cells. Parenteral administration with use of an infusion pump has been the major limitations. The studies revealed that the compliance decreased significantly with the increasing age. Deferasirox (Exjade®, ICL670) is an N-substituted bis-hydroxyphenyl-triazole was developed under a rational drug development program from over700 compounds. It represents a new class of tridentate iron chelators with a high specificity for iron with a plasma half-life of 8 to 16 hours. It is practical to administer the drug once a day orally and to maintain effective plasma levels. It is able to scavenge non-transferrin-bound "liable plasma iron, responsible for tissue damage". Two molecules of the drug form a stable iron complex which is excreted in the feces. Iron is chelated, both from the reticuloendothelial cells (RE cells) as well as various parenchymal tissues. The chelated iron is cleared by the liver and excreted through the bile. ICL670 produced a linear dose-dependent rise in net iron excretion, with wide variation seen at 40 mg/Kg/day while variation was much less in the dose of 20 mg / Kg. It is highly selective for iron and does not induce the excretion of zinc or copper.
Clinical Trials: In patients receiving 20 mg/Kg deferasirox the iron burden was essentially unchanged if the baseline LIC values were between 7 and ≤14mg Fe /g dw. While the patients receiving 30 mg/Kg the iron burden was reduced. In frequently transfused patients, defined as individuals receiving 2 to 4 units per month (or 7-14 ml/Kg/month) of packed RBCs, oral deferasirox once daily in dose of 20 mg/Kg led to maintenance of LIC, neutral iron balance, and stable serum ferritin levels. In another large phase 2 study, patients were randomized to receive once-daily deferasirox (10 or 20 mg / Kg; n=24 in both groups) or DFO (40 mg / Kg, 5 days / week; n=23) for 48 weeks. Decrease in liver iron concentration (LIC) were comparable in the deferasirox 20 mg/Kg/day and DFO groups; baseline values of 8.5 and 7.9 mg Fe / g dw fell to 6.6 and 5.9 mg Fe / g dw, respectively, by week 48. Hishet and his colleagues observed daily single dose of 20 mg/ Kg was well tolerated and was effective as an iron chelator. Most common adverse events with an apparent relationship to deferasirox were transient, gastrointestinal events were observed in 15.2% of patients that included abdominal pain, nausea and vomiting, diarrhea, and constipation. Skin rash was seen in 10.8% of patients. Deafness, neurosensory deafness, were observed infrequently. Mild transient dose dependent increase in serum creatinine was seen in nearly 1/3 of cases. There were no episodes of neutropenia, agranulocytosis or thrombocytopenia in any of the treatment groups. No adverse effects on growth or development in pediatric patients were observed. It should also facilitate patient compliance, a critical factor in effective patient management to maintain low iron burdens in patients requiring frequent blood transfusions. This drug meets all ideal properties of effective single oral iron chelators with a practical minimal side effects. Thus it serves as a sensational magic bullet of the century against hemosiderosis and hemochromatosis.

Nanomedicines - Self Nanoemulsifying Drug Delivery System (SNEDDS) and Nanosuspension for Oral and Parenteral Formulations in Cancer Therapy with Significant Impacts on Pharmacokinetics and Biodistributions

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Background: Mebendazole (Mbz), a highly lipophilic new anti-neoplastic agent (aq. Solubility: 0.7 µg/ml), is challenging for pharmaceutical formulation development. Aims: 1) To develop SNEDDS and Nanosuspension (NS) for oral and parenteral administrations in cancer therapy. 2) To characterize their pharmacokinetics (PK) and biodistributions (BD). 3) To establish the impacts of droplet/particle size on PK and BD of Mbz.
Methods: For SNEDDS, systematic approaches in excipient selections and SNEDDS-based nanoemulsion (NE) region identifications, using ternary phase diagram enabled the effective formulation optimizations. NS of sizes of 128-167, 250-253, 739-891 and 1552-1781 nm, were prepared using milling technique. The PK and BD of the nanoformulations were comparatively characterized in Sprague Dawley rat and Swiss athymic nu-mouse models.
Results: The SNEDDS-based NE of 35 and 143 nm significantly improved the oral bioavailability of Mbz, 228 and 120 times of that of unformulated suspension, respectively. With parenteral administrations of the NE, Mbz yielded prolonged half-life, 644 min vs 173 min, and sustained exposures in organs, especially in the lung, AUC of 12.4 vs 1.8 (hr*ug/g)/(mg/kg), from the cosolvent reference (CS). Droplet sizes (35 and 478 nm) impacted PK and BD, with an even higher concentration and a longer retention half-life with 35 nm than 478 nm NE. The Mbz from NS exhibited prolonged half-lives, 13-30 hr vs 3 hr of CS. The Vss were significantly larger, 1.19-1.69 L vs 0.06 L of CS, and peripheral V_2 were substantially larger, 24-49 L vs 0.4 L of CS. The size impacts were also demonstrated.
Conclusions: 1) SNEDDS of Mbz with droplet sizes of 35 – 478 nm were formulated for oral and parenteral administrations. 2) Nanosuspensions of Mbz with 4 particle sizes ranging from 128 to 1781 nm were developed. 3) Greater tissue distributions and slower in vivo dissolution of NS were key parameters responsible for the size-dependent PK distinction of Mbz. 4) Both nanoformulations offer potential merits of sustained and targeted cancer therapies, in lung and liver.

Akt Inhibitors: A New Strategy Targeting Long-Living HIV Macrophage Reservoirs

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Background: The infection of CD4+ T cells with Human Immunodeficiency Virus (HIV-1) is well-studied and typically leads to virally-induced cytolysis. In contrast, infected macrophages are able to uniquely evade the apoptotic effects of HIV-1 infection. These cells become infected early on and persist throughout viral pathogenesis. In addition, brain macrophages, or microglia, are thought to significantly contribute to the development of HIV-1 associated neurological problems. Since these cells seem to play a key role in HIV-1 pathogenesis, the study of the interactions between macrophages and HIV-1 is vital to understanding their role as persistent viral reservoirs. Here, the pro-survival effect of HIV-1 is presented as a mechanism for establishing these cells as viral reservoirs.
Methods: Primary human macrophages were infected with HIV-1 M-tropic YU-2 virus or a pseudotyped vector system HIV-GFP and used to perform experiments pertaining to cell survival and the role of PI3K signaling pathway. Miltefosine along with related PI3K/Akt inhibitors were used to antagonize the effects of HIV. Viral production and cell death were monitored.
Results: We previously found that HIV-1 infection and the expression of HIV-1 Tat protein were able to induce a pro-survival effect in primary human macrophages. Subsequent studies showed that these pro-survival effects could be attributed to the activation of the Akt survival pathway. A series of mechanistic studies further revealed that the modulation of the Akt pathway was key in establishing the extended survival phenotype in these cells following infection. Specifically, we observed a reduction in PTEN protein levels and a significant increase in Akt activity. Based on these data, the role of Akt inhibitors as an anti-HIV therapy was tested. Interestingly, Akt inhibitors and specifically Miltefosine were found to block the cytoprotective effect of HIV-1 infection and Tat expression in primary human macrophages. Furthermore, a drastic decrease in viral production was observed in HIV-1 infected macrophages following treatment with Miltefosine and related PI3K/Akt inhibitors.
Conclusions: Collectively, these data suggest that Akt inhibitors like Miltefosine may provide a means of targeting long-lived viral reservoirs and may offer further insight into novel therapeutic targets for anti-HIV therapy without concerns of viral resistance.

The urokinase system is a natural inducer of cancer cell drug resistance

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Background: Cell-bound urokinase converts serum plasminogen to plasmin. High membrane urokinase expression is associated with enhanced cancer cell metastasis formation and poor disease prognosis. Experiments were conducted to explore the relationship between urokinase activity and cancer cell drug sensitivity.
Methods: MCF7 Human breast cancer cells were cultured for 2 days in the presence or absence of plasmin, and subsequently exposed for additional two days to doxorubicin, paclitaxel, or tumor necrosis factor. Cell survival was determined.
Results: After culturing in the presence of plasminogen-containing human serum, 50% MCF7 cells were killed with 15 ng/ml doxorubicin. When cells were cultured in the presence of plasminogen-depleted human serum and thus blocked urokinase activity, as little as 5 ng/ml had an equal cytotoxic effect.
Addition of exogenous plasmin to plasminogen-depleted culture restored the drug resistance of cancer cells.
Blocking cancer cell's urokinase activity by anti-uPA antibody enhanced the susceptibility to doxorubicin toxicity.
The life preserving effect of plasmin is demonstrated to be mediated by the insulin-like growth factor (IGF). IGF is known to induce drug resistance in cancer cells. Blockage the IGF pathway abrogated the ability of plasmin to render cancer cells drug resistant.
Other apoptosis-inducing cancer drugs were tested in the presence or in the absence of plasmin. Plasmin-dependent drug resistance was observed in each case.
Conclusion:
1. Cancer cells utilize the urokinase system to acquire drug resistance.
2. Drug sensitivity is restored when the urokinase system is inhibited
3. Insulin-like growth factor (IGF) is identified as a mediator of this plasmin effect.

Magnetic Alginate Nanospheres: a Novel Vector for Targeted Drug Delivery

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Background: The explosive growth of nanotechnology in the last years has produces dramatic innovations in pharmacology. Precise targeting of drugs to diseased cells or locations within organs is considered the "magic bullet" in medical therapy, but it is not yet achieved by current drug delivery methods. Magnetic materials have been proposed for biomedical purposes to a large extent for several years. In this paper, the development, characterization, and *in vitro* test of alginate nanoparticles, embedding magnetite, which responds to externally applied magnetic fields, are presented.
Methods: Magnetite powder was obtained reacting iron(II) and iron(III) ions in an aqueous ammonia solution. Magnetite is then isolated from the reaction suspension and completely dissolved in an alginate solution. Alginate magnetic particles were realized by a homogenization process and reticulation with calcium ions. Such microparticles were characterized in terms of external morphology, size distribution, zeta potential, magnetic properties and drug release behaviour. *In vitro* testing was performed with NIH/3T3 and PC12 cells.
Results: Concerning the magnetic properties, magnetization curves show the typical trend of superparamagnetic materials. Important parameters, such as magnetic permeability (μ_r = 12.3) and magnetic momentum ($\mu = 2.25 \cdot 10^{-25}$ A·m^2), were derived by employing Langevin theory. A drug release of about 5-6 days was assessed usig albumin as protein model.
Nerve growth factor (NGF) loaded nanoparticles were tested on PC12 cells. Cells showed neuronal phenotype and developed neuritis with length strictly dependent on the distance from the nanospheres, fixed in a region of the Petri dish thanks to an external magnetic field. Finally, dynamic colture of NIH/3T3 cells incubated with fluorescein loaded magnetic nanoparticles definitely demonstrated the possibility to guide these nanovectors through a circuit simulation blood circulation.
Conclusions: 1) Alginate nanoparticles with a core of magnetite and filled with specific drugs were realized and fully characterized. 2) Magnetic nanospheres loaded with neurotrophic factors were able to trigger cell differentiation strictly depending on the distance of the release source. 3) The proposed system was successfully tested on dynamic cultures simulating blood circulation.

Treatment and relocation of wheat flour sensitized workers by an allergenic vaccine

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Background: Proteins binding IgE are natural components of wheat flour (WF) and the inhalation of particles during working is a major cause of WF occupational allergy (WOA) for bakers, pastry-makers or pizza-makers with workplace-related respiratory symptoms. They develop rhinitis and subsequently asthma with unfavourable consequences on quality of life, often being forced to give up their jobs and to claime compensation. Although drugs treatment could be useful for limited periods, attempts to realize a desensitization by continous administration of incremental doses of an allergen-specific vaccine (AV) were carried out in the clinical practice of recent years. An AV containing the most relevant fraction for WOA (water/salt soluble albumins and globulins) and standards related to this subcutaneous immunotherapy (SIT) are the remarks of the author's experience, whose evidence is proposed.
Methods: 43 subjects were AV-treated (39 M, 4 F, mean age 39 yr, range 23-67); bakers 27, pastry makers 13, pizza makers 4. All had a diagnosis of WOA with rhinitis and 60% suffered also of persistent mild asthma (prick test and specific-IgE positive, occupational challenge confirmed). WF-AV (Lofarma Laboratories, Milan) was characterized by SDS-PAGE and immunoblot; content of 14-17, 36 and 50 Kd proteins as major allergens was documented. The product was a retard preparation in aluminium hydroxide; an in-house reference standard (RAST Units, RU) and quality control were developed. SIT was always performed by the same single-patient management plan, without stopping the work, basing on a slow induction schedule of 4 months and a montly maintainance with 8000 RU for 4-7 yrs. Subjects were retrospectively interviewed after SIT by questionnaire and data were registered about symptoms, SIT-compliance, job conditions, global efficacy.
Results: SIT was tolerated without adverse effects; only 7 cases reported local reactions during starting months of SIT. Above all 34 subjects keepped up regularly his own job, 16 of them being at work after WF-SIT was discontinued from several yrs. Nobody claimed persistent asthma, while many sneezed again sometimes during work in the last year, but feeling rarely poor nasal occlusion or runny nose (see table). Only 9 subjects changed the activity at risk, because of better engagements elapsed or being retired for ageing; nobody for worsening of symptoms. The immunological paramethers (reduction of cell reactivity and of wheat-specific IgE) are reported elsewere.

Symptoms (n.workers)	Never (n.)	Sometimes (n.)	Often (n.)	Total (n.)
Eyes	14	20	0	34
Nose	4	24	6	34
Breath	10	20	4	34

Conclusions: 1) WOA may be treated by SIT with an AV. 2) A WF-AV was proved to be effective by a standardized protocol, duration at least 4 years. 3) Giving up a qualified job by bakers and similar exposed ones may be prevented, with economic and social advantages. 4) Compliance of SIT reduces the sensitization, improves symptoms and promote the work relocation in most of cases.

Management of Deep Space Neck Infection. Five Year Experience.

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Background: Deep neck space infections present as deep neck abscess or necrotizing fasciitis.They have severe potential for complication which can be life threatening. Aims: To find out predictive factors for more fulminant progression of infection.
Methods: We retrospectively analyzed medical data for patients surgically treated for deep neck space infection between 2002 and 2006. Our group of patients consists of 79 male and 53 female. We divided them in two groups: deep neck abscess and necrotizing fasciitis.
Results: 115 patients had deep neck abscess and 17 necrotizing fasciitis. Infection started with nondental origin (angina, epiglotitis, infected haemathoma) in 8 patients in first group and in 6 patients in second group. Among comorbidities Diabetes mellitus was the most common, others were prolonged treatment with corticosteroids, cirrhosis and chemotherapy.
Conclusions: Dental infections are the most common cause of deep neck abscesses and necrotizing fasciitis. Nondental origin is more likely to cause fulminant infection and necrotizing fasciitis. Diabetes mellitus was the most common comorbidity.

Zolpidem after brain Damage

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11 January 2009 marks the tenth anniversary of the awakening of Louis from his state of impaired consciousness 30 minutes after swallowing 10mg Zolpidem. In the past decade, Zolpidem has been effective in a multitude of patients with brain damage ranging from birth injury to traumatic brain injury to stroke and others. SPECT and PET studies in these patients show reactivation of brain metabolism in quiescent brain regions after injury, designated functional neurodormancy. MEG studies show that these suppressed neurodormant brain areas have a characteristic slow wave magnetic rhythm that normalises after Zolpidem, but not after placebo or other sleeping drugs such as Zopiclone. Neurodormancy arises as a physiological thread that is present in a multitude of unrelated brain pathologies, possibly due to a basic physiological protection mechanism that is initiated after brain damage. It forms a target for Zolpidem which re- activates neurodormant tissue and normalises clinical features that occur because of the suppressed neurodormant brain.

New Applications for Micro- and Nanoscaled Drug Delivery Systems

COESTER C

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Our group comprises four members located in the Pharmaceutical Technology of the Department Pharmacy, LMU-Munich, headed by Mr. Gerhard Winter. We are concerned with the pharmaceutical development of colloidal carriers for the targeted gene and tumor therapy by delivery of siRNA, RNA, plasmid DNA, and classical chemotherapeutics. The idea behind is to use simple and straight forward formulation approaches, which are easy to implement on a larger scale e.g. for the industrial production. Our colloidal carriers are produced solely out of biocompatible, biodegradable and low toxic materials like phospholipids, porcine gelatin and chemically modified derivatives of them. Besides our focus for advanced pharmaceutical quality, we chase for a high therapeutical efficacy in numerous clinical studies.
Our research is based on proper inventions and is one of the worldwide leading groups in the field of gelatin nanoparticles. The group's focus here is on high-end polymer and particle analysis with asymmetrical flow field flow fractionation (AF 4), novel *in-vitro* simulation models and the use of fluorescent or radioactive labeled nanoparticles for whole body *in-vivo* imaging. For several years the group holds a strong knowledge in the field of immunity based tumor therapy with CpG oligonucleotides delivered by nanoparticles made of gelatin and other natural polymers. A new matter of our group is the knockdown of disease - related genes by siRNA. This is anticipated to gain momentum with the aid of our newly established cell culture facility.
A further challenge we have recently taken is the development of acoustically active gas-filled microparticles, also called microbubbles. Microbubbles are known for over 15 years as contrast agents for the ultrasound diagnostic imaging. Recently, they have also proven promising carriers for drug and genes. After being injected, microbubbles can be visualized on the target site by using diagnostic ultrasound. Following, by increasing ultrasound intensity they can be destroyed and release their therapeutic load. Our research on microbubble carriers diverges in the fields of targeted therapy of solid tumors and targeted gene therapy.

The ADAM9 Disintegrin Domain (ADAM9D) Inhibits Platelet and Tumor Cell Adhesion to Subendothelial Matrix under Dynamic Flow Conditions

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Background: Members of the ADAM (*A D*isintegrin *A*nd *M*etallopeptidase) protein family are composed by a series of conserved protein domains including a disintegrin domain, which interacts with cell surface integrins. Adhesion to and extravasation through the endothelial lining of blood vessels is critical for tumor cells to establish metastasis. The objective of this work was to verify the effects of the disintegrin domain of ADAM9 (ADAM9D) on the adhesion of tumor cells (MDA-MB-231) and platelets to collagen type I, under flow to simulate shear conditions found in the circulation.
Methods: The recombinant ADAM9D was produced by cloning into a pGEX-4T-1 vector which was used transform *E. coli* AD494(DE3) cells. The synthesis of GST/ADAM9D was induced by IPTG (0.1mM, 4h). After purification on a Glutathione-Sepharose 4B resin, the ADAM9D was released from GST by cleavage with thrombin and further purified in a Benzamidine-Sepharose 4B column. MDA-MB-231 breast tumor cells labeled with cell tracker red were previously incubated with ADAM9D (5µM) or PBS (control) and then mixed with whole blood prepared labeled with calcein green. The mixture was perfused at a shear rate of $1500sec^{-1}$ in a flow chamber on a collagen type I-coated coverslip. Adhered platelets and cells in each field were differentially counted using the software Image J. The results were statistically compared with a two-way analysis of variance (ANOVA), followed by Bonferroni's significant difference post hoc analysis.
Results: Recombinant ADAM9D was able to inhibit about 50% of breast tumor cells and platelet adhesion to collagen type I, under flow conditions.
Conclusions: ADAM9D can be used as a tool for investigating the role of ADAMs in metastasis and cancer progression and for the design of selective inhibitors against the adhesion and extravasation of cancer cells.
Financial support: FAPESP, CNPq, INSERM.

***In situ* Photopolymerized Coatings for pH-Specific Drug Delivery from Pellets**

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Background: Coating of drug-containing pellets enables the delivery to be sustained or site-specific. As an alternative to the traditional coating procedure consisting of deposition of successive layers of preformed polymers, tailored coatings can be obtained by photopolymerization/ cross-linking of acrylic monomers on the pellet surface. Particularly, we have applied this new approach for preparing hydrogel coatings able to provide theophylline pellets with pH-dependent release rate.
Methods: Pellets of theophylline (20%), powdered cellulose (60%) and poly(vinylpyrrolidone) (20%) were obtained by extrusion-spheronization and then placed on a tray adapted to a vortex stirrer, pulverized with solutions of cross-linker and acrylic acid (AA) or AA:lauryl acrylate (LA) 88:12 molar ratio in ethanol:water medium and immediately irradiated with 366 nm UV-light. The coating process involved several sessions of pulverization and irradiation. Theophylline release was tested in 900 ml of 0.1N HCl or pH 7.4 phosphate buffer at 37ºC.
Results: The new photopolymerization process was particularly adequate for the coating of pellets since enabled: i) an easy and homogenous spraying of the monomeric solution on the pellets, ii) a quasi-instantaneous polymerization, and iii) a deep-thorough cure resulting in the formation of a well-structured network, of a high consistency but still flexible. No significant increase in pellet size (ca. 1 mm) was observed after coating. Non-coated pellets rapidly disintegrated and released all drug in few minutes. When only AA was used for the coating, the release process was sustained for 4 hours in 0.1 M HCl and finished in 2 hours in pH 7.4 medium. By contrast, coating with AA:LA mixtures and a cross-linker notably hindered the release at acid pH (only 50% released at 4 hours), without significantly compromising the fast delivery at neutral pH.
Conclusions: Photopolymerization/cross-linking of AA:LA on pellet surface enables site specific delivery as a function of pH and opens a wide range of possibilities for preparing tailored coatings adapted to specific triggering pH values. The coating conditions used in the present work could be easily adapted to industrial scale.

Authors disclosure statement:

Some information described in this abstract is the subject of patent applications filed by the University of Santiago de Compostela (ES 200600757).

Role of Drug Metabolism in the Development of Eplerenone (EP): A Lesson Learned from Spironolactone (SP).

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Background: EP is a highly selective aldosterone blocker approved in the US for the treatment of hypertension and heart failure. During development of the drug, extensive metabolism studies were necessary since another competitive aldosterone antagonist, potassium canrenoate (PC), that is structurally related to EP, was shown to be metabolized to potent mutagenic metabolites which appear to be associated with myelogenous leukemia. PC is a potassium salt of canrenoic acid formed from SP. However, SP did not produce these mutagenic metabolites because a thiol metabolite of SP inhibited formation of mutagenic metabolites from canrenoate.
Methods: In vitro metabolism studies of EP were carried out using human liver microsomes and cDNA-expressed CYP450 isozymes. Major metabolites were isolated and identified using MS and NMR. Inhibition of metabolism in human liver microsomes was examined in the presence of various chemical inhibitors at a concentration of 10 μM and with human CYP450 antibodies. Urinary and fecal metabolites were identified following oral administrating of [^{14}C]EP to healthy subjects at dose of 100 mg/person.
Results: Metabolic pathways of EP were 6β- and/or 21-hydroxylation by CYP3A4/5 and 3-keto reduction. The major metabolite identified was 6β-OH EP. There was no evidence for any alteration of the 9,11-epoxide ring or carboxy methyl ester. In contrast to canrenoate metabolism, no 6β,7β-epoxy metabolite, a precursor to mutagenic metabolites, was found with EP.
Conclusions: 1) Major metabolic pathway of EP was hydroxylation by CYP3A4/5. 2) Unlike PC, there was no 6β,7β-epoxide metabolite formed with EP. 3) Demonstration of stability of 9,11-epoxide ring and absence of 6β,7β-epoxy metabolite was essential in the development of EP.

Is there a magic bullet for prostate cancer?

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Background: Patients with advanced prostate cancer (CaP) frequently suffer from significant morbidity secondary to bony metastases. Therapeutic modalities that would eliminate or slow the growth of metastatic CaP and improve quality of life are of great interest. Although current docetaxel-based regimens offer superior survival advantage over previous standard therapies for men with castration-resistant metastatic CaP, this improvement resulted in an overall survival of only 18-19 months. Novel therapies with greater efficacy, either alone or in combination with docetaxel, are needed to continue to move the field forward to benefit afflicted men. The objectives of our studies were to characterize CaP metastases and test new treatment modalities.
Methods: Metastases of prostate cancer were obtained from patients who had died from advanced CaP and had consented to be part of the rapid autopsy program. There are two prevalent ways of selecting agents for combination therapy: utilize compounds that act by clearly distinct mechanisms (e.g. affecting either the tumor or microenvironment) or select compounds that affect different points in a specific signaling pathway critical to cancer progression. In preclinical testing, a model of experimental bone metastases was used whereby CaP tumor cells were injected directly in to tibiae of SCID mice. Animals were treated with various agents including docetaxel, zoledronic acid, RAD001, and the effects on tumor growth and bone remodeling were monitored.
Results: Our results clearly show that metastases of prostate cancer are phenotypically heterogeneous not only between different patients but also within a single patient. Our preclinical studies showed that while multiple agents are able to slow the growth of CaP in the bone, no single agent resulted in eradication of the tumor. Our further efforts have demonstrated that combinations of various agents inhibit the growth on CaP in the bone more than any single agent alone.
Conclusions: 1) CaP is an extremely heterogeneous disease; 2) Significant heterogeneity of CaP tumors makes treatment with a single agent unlikely to result in prolonged clinical responses; 3) Efficacy of drugs is affected by the site of metastases; 4) None of the tested agents was capable of eradicating the disease; and 5) Agents used in combination had more pronounced inhibitory effects.

Corticotrophin releasing factor as drug target for modifying dopaminergic system neuroplasticity in cocaine addiction

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Background: Cumulative evidence has demonstrated that stress plays a crucial role in cocaine addiction by enhancing the rewarding properties of the drug and inducing relapse during withdrawal. Corticotropin Releasing Factor (CRF) is one of the major effectors of stress in different structures of the reward circuitry, such the ventral tegmental area (VTA) and the amygdala. Aims: This paper explores the pattern of interaction between CRF, dopamine (DA) and glutamate (GLU) in different structures of the mesocorticolimbic circuitry during cocaine consumption. Understanding the neurochemical and cellular mechanisms involved in these processes would be useful in the development of new pharmacological strategies for the treatment of cocaine addiction.
Methods: A systematic literature review was conducted of animal and human research on the effects of stress through the CRF system. The interaction between CRF, dopamine and glutamate in VTA and the amygdala during repeated cocaine consumption and withdrawal were the focus of this review.
Results: In the VTA, stress-induced increase of CRF potentiates the activity of GLU transmission, which in turn increases the excitability of the DA system in cocaine-experienced animals. Blockade of CRF-R2 prevents stress-induced increase of synaptic plasticity in the DA system and relapse during withdrawal. In the amygdala, during early and late cocaine withdrawal, there is a progressive increase of CRF release that coincides temporally with behavioral anxiety during cocaine withdrawal and contributes to relapse. This increase in CRF activity potentiates GLU transmission, long-term potentiation, and synaptic plasticity. Selective antagonists of CRF-R1 are useful for preventing anxiety during withdrawal and reinstatement of cocaine seeking.
Conclusions: 1) Both CRF-R1 and CRF-R2 inhibitors may be useful for preventing withdrawal symptoms and relapse in cocaine addicts. 2) Bearing in mind the interactions between CRF, glutamate and DA in the different structures of the mesocortilimbic system, the use of combined pharmacological strategies involving all these neurotransmitters should be considered in the treatment of cocaine addiction.

BDNF and Its Intracellular Signaling Pathways as Drug Targets in Addiction

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Background: Craving and relapse, the most challenging aspects in the treatment of cocaine addiction, still lack an effective pharmacological treatment. Aims: This review examines the evidence supporting the involvement of brain-derived neurotrophic factor (BDNF) and its intracellular signaling pathways, mitogen-activated protein kinase (MAPK/ERK) and phosphatidylinositol 3-kinase (PI3-K), in the cerebral neuroplastic mechanisms in different structures of the mesocorticolimbic dopamine system underlying psychostimulant abuse and dependence, and also explores the possibility of using pharmacological inhibitors of these intracellular pathways as a new strategy to prevent cocaine craving and relapse during withdrawal.
Methods: A systematic literature review was conducted of animal and human research on BDNF and its intracellular pathways in acute and repeated cocaine consumption and withdrawal.
Results: Animal research demonstrates the involvement of BDNF, ERK and PI3-K in cocaine addiction. Repeated cocaine exposure and withdrawal induce an increase of BDNF and ERK in different cerebral areas, including the ventral tegmental area, nucleus accumbens (NAc) core and shell, amygdala and hippocampus. After repeated cocaine administration there is also a PI3-K up-regulation and a down-regulation in the NAc and prefrontal cortex, respectively. These molecular changes are associated with experimental measures of cocaine addiction, craving and relapse during withdrawal. Pharmacological blockade of the ERK a) prevents the acquisition of behavioral sensitization (increase of some behavioral responses), b) abolishes the association of environmental cues to cocaine, and c) decreases cocaine seeking during withdrawal. Pharmacological PI3-K inhibition after repeated cocaine prevents the expression of behavioral sensitization.
Conclusions: 1) BDNF modulates synaptic plasticity in cocaine addiction, especially during withdrawal, contributing to the transition from sporadic cocaine consumption to addiction and relapse. 2) ERK and PI3-K cascade inhibitors are potential therapeutic strategies to be further investigated in the context of addiction.

Anti-HIV And Anti-Cancer Drug-Drug Interactions

CORONA G

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The challenge in the treatment of human immunodeficiency virus (HIV)-related malignancies is represented by the need to maintain an adequate control of HIV infection while managing the malignant disease with anticancer therapy. Combination therapy with highly active antiretroviral therapy (HAART) and anticancer chemotherapy (CT) has been found to be effective and may contribute in reducing morbidity and extend survival of HIV patients with cancer. However, an increased toxicity of the treatment often limits the clinical benefits of HAART+CT combination leading to the interruption or dose reduction of both the anticancer and antiviral therapies, thus increasing the risk of viral rebound and tumor relapse. It was believed that such unexpected toxicity could be related to pharmacokinetic interactions between antiretroviral and antineoplastic drugs. Cytotoxic anticancer drugs have a narrow therapeutic index, and small variations in drug exposure consequent to metabolic interactions with the antiretroviral drugs may explain the increased toxicity observed during coadministration of HAART with chemotherapy. HAART consists of a combination of nucleoside analogue reverse transcriptase inhibitors (NRTIs), with protease inhibitors (PIs) and non-nuclease analogue reverse transcriptase inhibitors (NNRTIs). PIs, as well as many anticancer drugs are metabolized by the liver cytochrome P450 CYP3A4 isoform. The metabolic clearance of anticancer drugs sharing this common enzymatic pathway of PIs can be inhibited by concomitant administration of PIs. Moreover, PIs could also compete for cellular ABC transporter proteins such as P-glycoprotein (Pgp) or multi-drug resistance proteins (MRP), interfering with both biliary and renal excretions of the anticancer drugs. The current use in HAART of the new boostered formulations containing ritonavir (RTV), which is the most potent CYP3A4 and ABC transporter inhibitor among the PIs, increases the need for formal drug-drug interaction study to establish the extent and the clinical consequence of the pharmacokinetics interaction between PIs and anticancer agents. In this contest we have studied the effect of bostered lopinavir/ritonavir (LPV/RTV) on the pharmacokinetics of irinotecan (CPT11) in HIV patients with Kaposi's sarcoma. CPT11 is a prodrug that is activated in vivo by the liver carboxylesterase 2 to 7-ethyl-10-hydroxycamptothecin (SN38), a potent topoisomerase inhibitor. SN38 is inactivated to SN38 glucuronide (SN38G) by the UDP-glucuronosyl transferase 1A1 isoform (UGT1A1). In addition, CPT11 is oxidized to the inactive 7-ethyl-10-[4-N-(5-aminopentanoic-acid)-1-piperidino]-carbonyloxycamptothecin (APC) metabolite by CYP3A enzymes. Excretion pathways of CPT11 and metabolites in the bile and urine involve ABCB1 and ABCC2 transporters. This complex in vivo metabolism of CPT11 gives the opportunity to evaluate the effect of LPV/RTV on diverse metabolic pathways demonstrating that the inhibition of marginal oxidative metabolism route of CPT11, mediated by CYP3A4/5, could have dramatic consequence on the pharmacokinetics and pharmacodynamic profile of CPT11.

Docking calculations of heme derivatives on CYP2B4

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Cytochrome P-450 is a group of enzymes involved in the biotransformation of many substances, including drugs. These enzymes possess a heme group (1) that when it is properly modified induces several important physicochemical changes that affect their enzymatic activity. In this work, the five structurally modified heme derivatives 2–6
and the native heme 1 were docked on CYP2B4, (an isoform of P450), in order to determine whether such modifications alter their binding form and binding affinity for CYP2B4 apoprotein. In addition, docking calculations were used to evaluate the affinity of CYP2B4 apoprotein-heme complexesfor aniline (A) and N-methyl-aniline (NMA). Results showing the CYP2B4 heme 4- and heme 6-apoprotein complexes to be most energetically stable indicate that either hindrance effects or electronic properties are the most important factors with respect to the binding of heme derivatives at the heme-binding site. Furthermore, although all heme-apoprotein complexes demonstrated high affinity for both A and NMA, the CYP2B4 apoprotein-5 complex had higher affinity for A, and the heme 6 complex had higher affinity for NMA. Finally, surface electronic properties (SEP) were calculated in order to explain why certain arginine residues of CYP2B4 apoprotein interact with polarizable functionalities, such as ester groups or sp2
carbons, present in some heme derivates. The main physicochemical parameter involved in the recognition process of the heme derivatives, the CYP2B4 apoprotein and A or NMA, are reported.

The Interaction of the Organophosphorous Pesticide Methyl-Parathion with Serum Albumin by Fluorescence Spectroscopy

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Background: The aim of this work was to study the interactions of methyl-parathion (MP) with human (HSA), bovine (BSA) and fish (FSA) albumins by using the fluorescence quenching technique. The fish species used was the pacu (*Piaractus mesopotamicus*), a typical inhabitant of Brazilian rivers. MP is a pesticide still used in agriculture and fish hatcheries in many countries.
Methods: MP was purified from a commercial grade preparation by thin layer chromatography, using dichloromethane as the mobile phase. HSA and BSA were purchased from chemical company. FSA was isolated from the pacu serum by affinity chromatography. We excited the intrinsic fluorescence of the tryptophan in albumins and observed the quenching by titrating the protein solutions with MP. The Stern-Volmer graphs were plotted and the quenching constants were evaluated.
Results: The titration of HSA and BSA at 25ºC produced linear Stern-Volmer plots. At 37°C, the plot is still linear for HSA, but not for BSA. The titration of pacu albumin by the pesticide at 20ºC, 25ºC and 30ºC showed relevant slope deviations by the temperature changes. This behavior of Stern-Volmer plot of pacu albumin is analogous to the BSA. Examining the influence of the temperature at low MP concentrations on the plots, we found the occurrence of static quenching for the three albumins.
Conclusions: (1) The three studied albumins interact with MP to form complexes. (2) The pprimary binding sites for the pesticide on HSA and BSA are close to tryptophan residues 214 and 212, respectively. (3) Estimated association constants for HSA and BSA were, respectively: $3.07x10^4$ ($\pm1.2x10^3$) M^{-1} and $1.96x10^4$ ($\pm4.5x10^2$) M^{-1} at 25ºC; $1.08x10^4$ ($\pm2.0x10^2$) M^{-1} and $8.16x10^3$ ($\pm1.9x10^2$) M^{-1} at 37°C. (4) For pacu albumin, the Stern-Volmer constants were $1,19x10^4$ ($\pm3,4x10^2$) M^{-1} at 20ºC, $9,73x10^3$ ($\pm4,9x10^2$) M^{-1} at 25ºC, $9,37x10^3$ ($\pm4,4x10^2$) M^{-1} at 30ºC.

Small Peptides Challenge Thymidylate Synthase Dimerization and Inhibit Ovarian Cancer Cells Growth.

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Background: Ovarian cancer is the fifth most common cause of death from cancer in women. The standard first-line treatment is a combination of paclitaxel and carboplatin or carboplatin alone. In the case of progressive disease or drug resistance treatment with platinum, either alone or in combination, especially investigational compounds should be used. The mechanisms behind acquired resistance to cDDP and its derivatives are not clear yet, although it is evident that the process is multifactorial including, enhanced DNA repair. In the human ovarian carcinoma cell line A2780, a 3-fold-cDDP-resistance was associated with cross-resistance to the thymidylate synthase (TS) inhibitors 5-fluorouracil and to methotrexate, a 2.5-fold increase in TS level, and an increase in the intracellular pools of the TS cofactor 5, 10-methylentetrahydrofolate and of tetrahydrofolate. Our ultimate goal is to directly halt tumour progression and the development of drug resistance upon treatment with platinum derived drugs by inhibiting the protein regulatory function of monomeric TS through small molecule cellular perturbation.
Methods: To this aim we applied a multidisciplinary approach to identify new molecules that could bind to specific pocket at the protein interface. We included antipeptide design, site directed mutagenesis, tethering of thiol ligands at the protein interface, Mass spectrometry, x-ray crystallography.
The biological profile of the discovered inhibitors was characterized and the mechanism of action described.
Results: Four peptides among the ones identified showed inhibitory activity versus human Thymidylate synthase in the range of 10-400 μM. The most active and specific compounds identified showed effective TS inhibitory properties and cell growth inhibition properties against both sensible and resistant cancer cells.

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Magic Bullets - the Lantibiotic approach

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Background: Lantibiotics are antimicrobial peptides that frequently function at nanomolar concentrations by targeting the essential cell wall precursor, lipid II. Being gene-encoded, the lantibiotics can serve as biological templates for novel bioengineered antimicrobials. Bioengineering of lantibiotics first occurred in 1992, an event which prompted great optimism with predictions that lantibiotics might ultimately be regarded as the equivalent of primitive antibodies, potentially having constant and variable regions. However, unlike the mammalian immune system, the bacterium has no mechanism for generating a diverse population of peptides. Here we used molecular biology-based techniques to address this issue.
Methods: *nisA*, the gene encoding the structural peptides of the lantibiotic, nisin, were cloned into plasmid pCI372 and used as a template for error prone PCR-based random mutagenesis. The bioengineered genes were introduced the *L. lactis* NZ9800, which possesses all other genes required for nisin biosynthesis, to generate a bank of 8,000 strains producing different nisin derivatives. The bioactivity of a number of these lantibiotic producers and the specific activity of the most potent of the peptides produced was quantified.
Results: The approach described led to the identification of a nisin-producing strain with enhanced bioactivity against *Streptococcus agalactiae* resulting from a residue change in the middle 'hinge' region of the peptide (K22T). Further 'hinge'-specific mutagenesis took place, resulting in the identification of additional derivatives, most notably N20P, M21V and K22S, with enhanced bioactivity and specific activity against gram-positive pathogens including *Listeria monocytogenes* and/or *Staphylococcus aureus*. In a number of cases this antimicrobial activity was species specific.
Conclusions: The identification of these derivatives represents a major step forward in the bioengineering of nisin, and lantibiotics in general, and confirms that peptide engineering can deliver derivatives with enhanced antimicrobial activity which could be regarded as being 'Magic Bullets' with respect to the targeting of specific problematic spoilage and pathogenic microbes.

Aspirin resistance myth or reality?

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Background. The concept of aspirin resistance is controversial with reports of clinical studies showing an association with outcome in cardiovascular patients and other researchers claiming that it doesn't exist. This study was designed to determine if the recent trend to use low dose enteric-coated aspirin may play a role in the phenomenon of aspirin resistance
Methods. 71 healthy volunteers were administered a low dose aspirin preparation and in a subsequent study 144 cardiovascular patients on low dose aspirin were recruited. The effect of aspirin was monitored by arachidonic acid-induced platelet aggregation, VerifyNow® and serum thromboxane. The definition of aspirin resistance was assay dependent.
Results. None of the healthy volunteers were considered to be aspirin resistant as they showed complete inhibition to at least one aspirin preparation. However there was variation in the response to different preparations of aspirin as determined by serum thromboxane. In particular the response to enteric-coated aspirin was not as good as that to plain aspirin ($P<0.001$) and this was exacerbated by weight with a poorer response among heavier subjects ($P<0.001$). Among the patients the incidence of aspirin resistance was between 13% and 21% depending on the assay ($P>0.05$) although each assay detected different patients and there was little agreement between the assays, however, when patients were re-tested with a reminder to take their medication only 2% were considered to be resistant.
Conclusions. Aspirin resistance appears to be a rare phenomenon. In most cases it is due to poor compliance. In many of the remaining patients the lower bioavailability of enteric-coated aspirin, especially when combined with higher weight results in a significant reduction in the dose/kg that the patient is exposed to. Thus, the phenomenon is best described as aspirin non-response rather than aspirin resistance. However, it is still clinically relevant as aspirin is only effective if the patient takes it in sufficient quantities.

Mechanisms of anti-CD20 immunotherapy: Why Type II mAb are better

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Background: Rituximab is an anti-CD20 monoclonal antibody (mAb) that was the first of its kind to be approved by the FDA. It is now part of the standard treatment for many B cell malignancies and is finding utility in a range of autoimmune diseases where depletion of normal B cells, although not as yet explained mechanistically, appears highly beneficial. Treatment is not always successful however and not surprisingly the pursuit of improved reagents to replace rituximab is intense. Anti-CD20 mAb can be sub-divided into Type I (like rituximab) or Type II (tositumomab-like) based on their ability to redistribute CD20 molecules in the plasma membrane and activate various effector functions such as complement. Therefore, we wished to address whether other (Type II) anti-CD20 mAb might be more effective than rituximab in deleting B cells.
Methods: To compare Type I and II anti-CD20 mAb directly in vivo and maximize Fc effector function, we selected and engineered mAb with the same mouse IgG2a isotype and assessed their B cell depleting activity in two different strains of human CD20 transgenic mice. The ability to deplete peripheral blood and secondary lymphoid organs was assessed by flow cytometry and immunohistochemistry.
Results: Despite being the same isotype, having similar affinity, opsonising activity for phagocytosis, and in-vivo half-life, the Type II mAb tositumomab provided substantially longer depletion of B cells from the peripheral blood compared with the Type I mAb rituximab (Rit m2a), and 1F5. This difference was also evident within the secondary lymphoid organs, in particular the spleen. Failure to engage complement did not explain the efficacy of the Type II reagents, since Type I mAb mutated in the Fc domain (K322A) to prevent C1q binding still did not display equivalent efficacy. We have recently determined the likely mechanism through which Type II mAb outperform Type I mAb and will present this data.
Conclusions: These results provide strong support for the development of Type II anti-CD20 mAb for the treatment of B cell diseases and expect that their lack of complement engagement should reduce the toxic side effects often associated with the use of rituximab and other Type I reagents. This work also provides insight into how mAb to other targets might be optimized for better therapeutic efficacy.

Authors' disclosure statement:
The authors declare no competing financial interests.

Allergy vaccines: dreams and reality

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Allergy, asthma, and atopic eczema derive from deregulated immune responses against innocuous antigens. The incidence of atopic diseases is affecting around 30% of the population in industrialized countries and has become a relevant socio-economic burden for the modern society. Although much progress has been achieved in the development of efficient symptomatic treatments for allergic diseases, the only curative treatment remains allergen-specific immunotherapy (SIT). In contrast to classical vaccines which elicit strong host immune responses after one of a few injections, SIT require a long treatment time of 3 to 5 years to confer some protection. During the last year, three promising allergy vaccination concepts [RPE 04 (Curalogic); GT-14 (ALK-Abelló); TOLAMBA (Dynavax)] have been put on hold in Phase III due to the lack of efficacy. The reality is that "allergy vaccines" achieve beneficial effects through immunomodulation which takes a long time to establish, the dream would be to develop "true" allergy vaccines able to cure the disease with a few injections. We have engineered modular antigen translocation vaccines (MAT) for direct intracellular targeting of the MHC class-II presentation pathway aimed to increase the efficacy of antigen presentation. MAT vaccines administrated directly into the lymph node in mouse models of allergy were able to completely protect the animals from anaphylactic shock in a very short time. Intranodal administration of low doses of pollen extracts to allergic patients was able to confer a long lasting protection after only three injections administrated over a period of eight weeks only. Now we are combining a MAT-Fel d 1 vaccine with intranodal administration in a Phase I clinical study ready to start at beginning of July 2008. The combination of a new route of administration with a direct targeting of the MHC class-II presentation pathway has to potential to realize the dream of curing allergy in a few weeks.

Naked Models of Compound I of Heme Enzymes

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Background: High valent iron porphyrins at the formal Fe(V) oxidation level are known as Compound I intermediates, invoked in the oxidation pathways catalyzed by heme proteins and by their synthetic model compounds.[1] A dominant feature of these processes is the dramatic change in product patterns and selectivities that Compound I may exhibit under the influence of reaction conditions. In this context, gas phase studies may have a great potential in revealing the intrinsic behaviour of this key intermediate.
Methods: Electrospray ionization (ESI) in combination with Fourier transform ion cyclotron resonance mass spectrometry is used to characterize the gas phase reactivity of high-valent oxoiron intermediates formed by two distinct procedures. In the first one, the oxoiron(IV) porphyrin cation radical intermediate, $[(TPFPP)^{\cdot+}Fe^{IV}=O]^+$ (TPFPP = 5,10,15,20-tetrakis (pentafluorophenyl)porphinato dianion) ion is prepared in solution by the reaction of the iron(III) porphyrin chloride, $[(TPFPP)Fe^{III}]Cl$, and H_2O_2 in methanol and then transferred to the gas phase by ESI.[2, 3] Alternatively, the naked core of Compound I is synthesized by the reaction of iron-protoporphyrin-IX (heme) ions, $[(PP\text{-}IX)Fe^{III}]^+$, with ozone in the gas-phase.
Results: The formation of the oxo-complex, described as a gaseous iron(IV)-oxo protoporphyrin IX radical cation species, $[(PP\text{-}IX)^{\cdot+}\ Fe^{IV}=O]^+$, allows a viable entry to a species that proves to be elusive in solution where it evolves presumably by activating the rapid growth of degradation products.[4]
The reactivity properties of the so-obtained high valent oxo iron intermediates with exemplary biologically active molecules and model compounds of naturally occurring substrates (L) of hemeproteins are reported. The reaction efficiencies, which measure the % fraction of reactive collision events, appear to increase with the oxophilic character of the active site of L (C<N<S<P).
Conclusions: Gas phase studies may contribute to elucidate complex mechanisms in enzyme chemistry providing highly simplified models.
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Prognostic value of total AgNOR area/Nucleus area per cell in urinary bladder carcinoma via two-dimensional image analysis

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Background: Traditional criterions are not sufficient to predict accurately the recurrence of transitional cell carcinoma of the urinary bladder. Therefore, we aimed to evaluate comperatively the nucleolar organizer region-associated proteins (AgNORs) via total AgNOR area/nucleus area (TAA/NA) for each cell, and as a prognostic parameter, comparing with total AgNOR number/nucleus (TAN/N), in TCC of urinary bladder.
Methods: Tumor tissues of 20 consecutive cases of male bladder cancer patients were divided into two groups as middle differentiated (LG) and high grade (HG). The extra-tumoral tissue (ETT) samples of 10 males served as control group. A second control group (HC) consisted of five healthy and normal bladder tissue samples. The 3 µm of sections from each paraffin embedded tumoral, extra-tumoral and normal tissue samples served as patient and control groups. After deparaffinization and rehydratation steps, AgNORs silver stained. Instead of Giemsa stain, we used Hematoxylin for contra staining. The images of the 100 analyzable nuclei from each tissue sample transferred by means of a video camera and video capture card from microscope and recorded onto a computer. Software was prepared in Delphi language for analysis.
Results: Mean (E+02) TAA/NA values were significantly different between all groups (p values < 0,001). While the mean TAN/N values of the groups were able to distinguish only malign samples from normals (p values < 0.05). The data is given in the table.

Table . TAN/N and TAA/NA values of control and patient groups

Groups	N	n	Mean TAN/N +S.D. (%)	Mean TAA/NA + S.D.(mean E+02)
HC	**5**	**500**	**1.31 + 0.57**	**6.97 + 2.80**
ETT	**10**	**1000**	**1.52 + 0.77**	**5.70 + 1.82**
LG	**10**	**1000**	**1.56 + 0.77**	**7.80 + 3.22**
HG	**10**	**1000**	**1.56 + 0.82**	**9.24 + 3.88**

N, number of tissue samples; n, number of examined nuclei.

Conclusion: As a new approach the evaluation of mean TAA/ NA per cell has a great potential to be a prognostic parameter and it has been found more sensitive and more accurate than the TAN/N determination. Therefore, further evaluation of big patient series will be useful.

Do we need a smaller Magic Bullet for combating Staphylococcal infections? : Mechanism of vancomycin-resistance in vancomycin-intermediate *S. aureus*

CUI L

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Since our reports on the discovery of vancomycin-intermediate *Staphylococcus aureus* (VISA) and heterogeneously vancomycin-resistant *Staphylococcus aureus* (hetero-VISA) in 1997 (JAC, 1997' 40:135-6 and Lancet, 1997' 385:1670-3), we have published a dozen serial papers on the molecular mechanism of vancomycin resistance in VISA, mainly in JAC (98' 42[2]:199-209 and 42[3]:315-20), AAC (2000' 44:1176-1185), Lancet (2001' 357: 1225-40), JCM (2003'41:5-14), Mol Micro (2003' 49;807-21), Lancet (2004' 364: 565-6) and AAC (2005' 49: 3404-13, 2006' 50' 428-38 and 2008' 52: 45-53). By performing the above serial studies, we have elucidated the molecular mechanism of vancomycin resistance: VISA resists vancomycin via a thickened cell wall, which in turn results in a novel mechanism of "clogging" of the peptidoglycan mesh, whereby incoming vancomycin molecules are trapped in the thickening cell wall, preventing them from reaching the cell membrane. Quantitative measurement and mathematic analysis revealed that the "clogging" is related with the bigger molecule of vancomycin. Moreover, in a recent study with daptomycin-nonesusceptible *S. aureus*, we found a strong positive correlation between vancomycin and daptomycin resistance in VISA, and the result suggests the thickened cell wall acts as a common obstacle to daptomycin and vancomycin penetration. Even though daptomycin does not bind peptidoglycan to form subsequent physical barriers within the cell wall, it might be hard for daptomycin, with as big a molecule as over 1620, to penetrate smoothly through the cell wall when the cell wall become as thick as that of VISA. Taking all together, we propose that development of new antibiotics with smaller molecular size than that of vancomycin and daptomycin may be a new potential to overcome the vancomycin- and daptomycin-resistant Staphylococci infections.

Fundamental Understanding on Interactions of Bisphosphonates with Bone (by Use of Different Techniques, Including Computational Modelling).

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Background: Computational modelling of BP-bone interactions is rare and hence there is very little to link the molecular structure of BP with its potency. Aims: 1) To determine stability constants of BPs with metal ions;. 2) To investigate interactions between BPs and a bone surface. 3) To model computationaly the interactions of BPs with a bone surface.
Methods: Raman spectroscopy (RS) was used to study the interactions of bovine bone, hydroxyapaptite (HA), and $CaHPO_4$ with HEDP (a reference compound in scale of potency). Generalised AMBER Force Field (GAFF) was used to model over 50 BPs ($C(R_1)(R_2)(PO_3^{2-})_2$) and the interaction of MDP ($R_1 = R_2 = H$), HEDP ($R_1 = OH$, $R_2 = CH_3$), APD ($R_1 = OH$, $R_2 = (CH_2)_2NH_3^+$), ALN ($R_1 = OH$, $R_2 = (CH_2)_3NH_3^+$) and NER ($R_1 = OH$, $R_2 = (CH_2)_5NH_3^+$) with the (001), (010) and (100) faces of HA. Metal-BP stability constants were established by voltammety and potentiometry; this allowed us to conclud on mode of complexation at blood plasma pH. Linnear Free Energy Relationships generated allowed us to predict stability constants of radioactive elements with BPs; potential use in bone cancer therapy.
Results: The Raman spectra of the products from the reaction of 0.5 M HEDP solution with bone, HA and $CaHPO_4$ could be considered virtually identical meaning that the complexes of HEDP formed were the same. From GAFF, all BPs react with HA exothermically (10 and 20 kcal mol^{-1}). With a dielectric constant of $78\varepsilon_o$ and $<10\varepsilon_o$, non-bonded and electrostatic interactions dominate, respectively. The order of increasing interaction with HA, MDP < HEDP < APD < ALN > NER, accords with the observed *in vivo* order of pharmacologic activity (potency) and parallels the increase in molecular volume up to ALN; the side chain of NER fails to interact fully with HA. There is no significant difference in the structure of the BP-HA complex if BP is a mono- or bis-protonated. Relationships established from metal equilibria allowed prediction of radiisotops'interactions with BPs.
Conclusions: 1) HA can be substituted for bone in fundamental studies of BP-bone interactions. 2) The consonance between experimental and molecular modelling results suggests that GAFF may be a useful tool to aid in the design of novel BP ligands. 3) From stability constants it was concluded that side chain of BPs is not involved in complexation with metals at blood plasma pH.

Prostones as ClC-2 Channel Activators for Treatment of Diseases and Disorders.

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Background: Prostones are a class of bicyclic fatty acids. One of these, lubiprostone is approved for the treatment of chronic idiopathic constipation (CIC) and irritable bowel syndrome with constipation (IBS-C) in the United States.
Methods & Results: Lubiprostone activates Cl^- currents, thus increasing salt and water flow in the small intestine. Electrophysiological studies of epithelial tissues show that Cl^- currents are increased by low concentrations (<100 nM) lubiprostone. These Cl^- currents persist with knockdown of the cystic fibrosis transmembrane regulator (CFTR), another epithelial Cl^- channel. However, the lubiprostone-activated Cl^- currents are ablated by knockdown of ClC-2. Recombinant human ClC-2 is activated in a dose-dependent manner by lubiprostone (EC_{50} = 20 nM). Recombinant human CFTR was not activated at concentrations as high as 1 µM. Lubiprostone increased single 3-4 pS Cl^- channel activity of both human and *Xenopus* ClC-2 in the apical membrane of the cells, at concentrations <100 nM. This is consistent with apical membrane localization of ClC-2 in T84 and Caco-2 human intestinal cell lines using nystatin permeabilization approaches. This is also consistent with animal studies showing increased salt and water transport into the intestinal lumen with lubiprostone. ClC-2 activation by lubiprostone does not involve increases in intracellular cAMP or Ca^{2+}, activation of prostaglandin receptors or phosphorylation by PKA. In Caco-2 cells and porcine ileum ClC-2 is present at the tight junctions. In porcine ileum, lubiprostone appears to promote repair of epithelial barrier function impaired by ischemia, accompanied by movement of ClC-2 to the tight junctions, a mechanism which may contribute to the treatment of IBS-C.
Conclusions: 1. Various studies have demonstrated that lubiprostone is a Cl^- channel activator that activates ClC-2 in the apical membrane of intestinal cells. 2. ClC-2 activation may be beneficial in diseases and conditions where tight junction integrity is compromised. 3. ClC-2 activation by prostones is a useful therapeutic tool in GI disorders. Supported by a grant from Sucampo Pharmaceuticals Inc.

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according to registration: Curkovic Perica

Auxins: Effects on Bacteria and Tumours

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Background: Phytohormones auxines are a group of molecules involved in mediating a number of essential plant growth and developmental processes. These plant hormones are not only synthesized by higher plants, but also by lichens, mosses, fungi and bacteria. Furthermore, not only bacterial species that live in soil and/or interact with plants produce phytohormons. Some human-associated and pathogenic bacteria produce auxins, too. It was shown that indoleacetic acid (IAA), as the most widespread natural auxin present in most living organisms, can trigger alterations in the main metabolic pathways of bacterial, yeast and human cells.
Methods: Auxins indole-3-acetic acid (IAA) **a**nd indole-3-butyric acid (IBA) were used to treat phytoplasmas and tumours. Phytoplasma-infected plants were maintained on Murashige and Skoog medium supplemented with IAA (11 or 57µM) or IBA (2.5, 4.9, 9.8 or 19.7 µM). Randomly chosen shoots from each treatment were tested for the presence of phytoplasmas by nested polymerase chain reaction amplifying highly conserved phytoplasmal 16S rRNA gene. Cytotstatic activity on tumour cells was assessed on 5 human cell lines, which are derived from 4 cancer types: MCF-7 (breast carcinoma), SW 620 (colon carcinoma), HCT 116 (colon carcinoma), MOLT-4 (acute lymphoblastic leukemia), H 460 (lung carcinoma). IAA and IBA were added to the cell culture medium in five, 10-fold dilutions (10^{-7} to 10^{-3} M) and incubated for 72 hours. The cell growth rate was evaluated by performing the MTT assay, which detects dehydrogenase activity in viable cells.
Results: The mechanism of auxins effect on phytoplasmas (mycoplasma-like organisms, economically important plant pathogenic bacteria), involves changes in plant-host metabolism and gene expression. It was shown that phytoplasma-free plants can be obtained upon treatment of infected plants with IBA, while treatment with IAA induced recovery of plants, but the pathogen was still present in the plant tissue. IAA also inhibited the growth of tumor cells (IC_{50} ≈ 500 µM), whereby the breast cancer cell line MCF-7 was the most sensitive cell line. Thus both auxines could be further evaluated as novel anticancer therapeutics.
Conclusions: Auxins exhibit both antibacterial and antitumour activity.

Spontaneous Formation of L-*iso*Aspartate and Gain-of-Function in Fibronectin

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Isoaspartate formation in extracellular matrix (ECM) proteins, by aspartate isomerization or asparagine deamidation, is generally viewed as a degradation reaction occurring *in vivo* during tissue aging. For instance, non-enzymatic isoaspartate formation at RGD-integrin binding sites causes loss of cell adhesion sites, which in turn can be enzymatically "repaired" to RGD by protein-L-*iso*Asp-*O*-methyltransferase (PIMT). We show here that isoaspartate formation is also a mechanism for ECM activation. In particular, we show that deamidation of Asn^{263} at the Asn-Gly-Arg (NGR) site in fibronectin N-terminal region generates an $\alpha v\beta 3$-integrin binding site containing the L-*iso*DGR sequence, that is enzymatically "deactivated" to DGR by PIMT. Furthermore, rapid NGR-to-*iso*DGR sequence transition in fibronectin fragments generates $\alpha v\beta 3$ antagonists (named "isonectins") that competitively bind RGD-binding sites and inhibit endothelial cell adhesion, proliferation and tumor growth. Time-dependent generation of *iso*DGR may represent a sort of molecular-clock for activating latent integrin binding sites in proteins.

Isoaspartate-glycine-arginine (isoDGR): a new tumor vascular targeting motif

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Various peptides containing the NGR motif have been discovered by selecting peptide-phage display libraries in tumor-bearing animals. These peptides can home to tumor neovasculature by binding aminopeptidase N (CD13), a marker of angiogenic vessels. We have previously demonstrated that NGR peptides can be exploited for ligand-directed delivery of cytokines, e.g. TNF and interferon-γ, to CD13-positive tumor blood vessels. One of these conjugates, made of CNGRC fused to tumor necrosis factor-a (NGR-TNF), is now under investigation in phase II clinical studies for cancer treatment, highlighting the value of CD13-targeting peptides in drug development. We have also found that NGR can rapidly convert to isoDGR and DGR by asparagine deamidation. In vitro and in vivo studies have shown that isoDGR, but not DGR, can bind $\alpha v\beta 3$ integrin in the tumor neovasculature. Pharmacological studies performed in tumor-bearing animal models showed that low doses (picograms) of NGR-TNF or isoDGR-TNF fusion proteins are sufficient to induce anti-tumor effects, by virtue of a targeting mechanism, when administered alone or in combination with chemotherapy. Considering that CD13 and $\alpha v\beta 3$ integrin are markers of angiogenic blood vessels, natural or synthetic polypeptides containing the NGR or the isoDGR motif may be exploited as ligands for targeted delivery of cytokines, nanoparticles, genes or imaging compounds to angiogenic vasculature in tumors.

Disulfiram as a New Promising Anticancer Drug

CVEK B

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Proteasome: Proteasome is a giant protease responsible for degradation of about 90% of cellular proteins. Key signaling proteins are activated through proteasome processing (e.g. nuclear factor-κB); moreover, proteasome plays an important role in regulation of transcription and other crucial cellular events. Curiously enough, the proteasome has become an attractive target in cancer therapy as first-in-class proteasome inhibitor bortezomib (Velcade) was approved for clinical use against multiple myelome and mantle cell lymphoma. At the present time, there are plenty of ongoing clinical trials of bortezomib in various cancers.
New use for old drug: An old drug against alcoholism, disulfiram (Antabuse), can create metal complexes which are potent proteasome inhibitors (Cvek & Dvorak *Drug Discov Today* 2008). Even further, disulfiram taken with zinc gluconate led to clinical remission in a patient with metastatic melanoma (Brar et al. *Mol Cancer Ther* 2004). There are substantial advantages of such "repurposing" of disulfiram: The drug (just because it is old) is cheap, safe, and is able to enter phase II clinical trials directly (Chong & Sullivan *Nature* 2007).
JAMM domain inhibition: Molecular mechanism of such disulfiram-mediated proteasome inhibition is putatively based on interaction between the metal complexes and key zinc subunit (JAMM domain protein Poh1) of the proteasome (Cvek & Dvorak *Curr Pharm Des* 2007). This approach could be an attractive strategy for future proteasome targeting in patients (Gallery et al. *Mol Cancer Ther* 2007).
Conclusions: Although there are two clinical trials of disulfiram as an anticancer agent listed in US National Cancer Institute database, they are not proteasome-focused (as disulfiram has other abilities to suppress cancer). Thus, potencial anticancer effects of disulfiram in the patients should be carefully evaluated in light of current knowledge on proteasome inhibition.

Behavioural and cellular consequences of excessive amounts of the powerful brain chemical dopamine

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Background: Parkinson's disease is associated with severe degeneration of nigrostriatal dopamine producing neurons. Pharmacological dopamine replacement with its synthesis precursor, Levodopa, remains the most effective treatment for Parkinsonism. However, long term treatment with Levodopa leads to the emergence of abnormal involuntary movements known as dyskinesia. Here we demonstrate that high doses of levodopa, leading to excessive amounts of dopamine in the brain, could be toxic to neuronal cells.
Results: Recently, we have demonstrated in cell cultures model and in rats striatum slices that activation of D1 receptors could regulate functions of structural proteins such as the microtubule associated protein tau. Whether dysfunctions in cytoskeletal-associated proteins are associated to Levodopa-induced dykinesia is unknown. This study investigated, in striatal neurons of 6-hydroxydopamine lesioned rats receiving or not Levodopa therapy, the expression and phosphorylation levels of tau. Our results demonstrated that, whereas dopamine depletion was without effects, intermittent administrations of Levodopa were associated with dyskinetic behaviours and had profound influence on tau phosphorylation.
Conclusions: These data provided novel evidence that alterations in cytoskeletal constituents could play a role in the emergence of motor dysfunctions associated with dopamine replacement therapy.

Peritoneal Transport Dynamics of Icodextrin and Its Influence on The Membrane Permeability In Vitro

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Background: Glucose polymer (icodextrin) is used during peritoneal dialysis for development of an effective ultrafiltration. It has been proposed also as a carrier solution of antineoplastic drugs and a factor to reduce of adhesion formation during intraperitoneal chemotherapy. The aim of the presented study was the comparative analyses of the peritoneal transport dynamics of icodextrin in the different conditions and its effects on the transfer of small and large molecules across peritoneal membrane intact and chemically injured in vitro. The rabbit parietal peritoneum, modified Ussing chamber and mathematical model of mass transport were used to calculate the diffusive permeability coefficient (P) in $cm \times s^{-1}$.
Results: Icodextrin (7.5 g/dL) peritoneal transport in vitro, in the control conditions, directed from the interstitial (I) to the mesothelial (M) side of the membrane changes with time, but in the opposite direction is constant. Asymmetry of glucose polymer diffusion is observed: I→M predominates over M→I. Fluid stirring intensification and chemical injury by sodium deoxycholate enhance bidirectional transfer of icodextrin. M→I transfer of icodextrin, but not I→M is restricted more by tissue barriers than stagnant fluid layers. Glucose and gentamicin intensify (M→I direction only) of the examined parameters, but methylglyoxal does not change the P values. Icodextrin modifies the transport dynamics of low and high molecular weight solutes across intact and mesothelium denuded peritoneum. For example it induces asymmetry of uric acid and albumin transport. I→M transfer dominates M→I transfer of uric acid. In contrast, in albumin transport M→I was higher than I→M. For the injured peritoneum the decrease in time of the bidirectional uric acid and urea transport, caused by icodextrin, is noted. Glucose polymer periodically augments and next diminishes gentamicin transfer directed from I to M side of the peritoneum. The decrease of antibiotics transport occurs also to the opposite direction.
Conclusion: The results may be important for the clinical point of view. The increase of icodextrin absorption from the abdominal space to blood, during peritoneal dialysis, may have a negative impact on the efficacy of this therapy. In contrast, asymmetry of uric acid and albumin transperitoneal transport induced by icodextrin, may be observed in vivo, seems to be beneficial.

Dopamine transmission and the search for the ideal antidepressant drug

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Background: We examined (i) the relationship between antidepressant treatment and two stress regimes, Chronic Mild Stress (CMS) and Daily Restraint Stress (DRS), which differ in their ability to affect sensitivity to dopamine agonists and (ii) the effect of different antimanic drugs on antidepressant-induced dopaminergic supersensitivity. CMS, a model of depression, is based on the exposure to a variety of mild stressors (e.g. short periods of crowding, isolation, food and water deprivation etc.), DRS consists of daily sessions of 1 hour immobilization, and they result either in a decreased or in an increased sensitivity to dopamine agonists, respectively.
Methods: Male Wistar or Sprague-Dawley rats were used. No-stress, CMS and DRS subjects were daily treated with the antidepressant desipramine. Stress and drug administration were commenced simultaneously and carried out for 7 weeks. The antimanic drugs lithium, carbamazepine and valproate, in three different experiments, were coadministered with the antidepressant imipramine for 3-4 weeks. After the chronic drug/stress treatments, the subjects were challenged with the dopamine D2-like agonist quinpirole and the motor response was measured.
Results: As expected, antidepressants and DRS potentiated while CMS reduced the motor response to quinpirole. Desipramine countered stress effect in both cases. Carbamazepine, but neither lithium nor valproate, prevented the imipramine-induced potentiation, an effect likely due to liver enzymatic induction.
Conclusions: 1) Countering stress effect on dopaminergic sensitivity, rather than just inducing a potentiation, might be what matters to attain the antidepressant therapeutic effect. 2) The failure of antimanic treatments to prevent dopaminergic supersensitivity might reflect their poor efficacy in treating antidepressant-induced manic switches, thus providing the basis for a possible model of antidepressant-induced mania.

Ras as a target for exploratory study of monoterpene perillyl alcohol intranasal administration in patients with recurrent gliomas

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Upon completion of his master´s degree in medical genetics at the Federal Rio de Janeiro University in 2003, Dr Fonseca joined the Laboratório de Patologia Celular – Departamento de Biologia Celular, of the Federal Fluminense University where he directed research in basic câncer research with translational research. This led to the development of invention of new approach for delivery drugs for malignant gliomas. He concludes PhD Thesis "Exploratory study of monoterpene perillyl alcohol intranasal administration in patients with recurrent gliomas" in 2007. Dr Fonseca pioneered a new technology, intranasal administration of monoterpene perillyl alcohol. Intranasal administration of perillyl alcohol is not only an unique delivery method, but the use of perillyl alcohol as a novel chemotherapeutic agent inhibiting the ras pathway is extremely exciting. His results have demonstrated that intranasal perillyl alcohol will soon be able technique in armamentarium against malignant gliomas (Da Fonseca et al 2006; Da Fonseca et al 2006; Da Fonseca et al in press). It is expected that this work will lead development of methods for combine drugs with different therapeutic targets.

Methotrexate (MTX) Induces Permanent Growth Arrest in Human Adenocarcinoma Cells

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Background: MTX is a classic antifolate successfully used in cancer chemotherapy for nearly 60 years. MTX was found to induce apoptosis, senescence or differentiation as treatment outcomes in various cancerous cell types. A major hurdle in MTX cytostatic efficacy is resistance to the drug. The present study is aimed at characterization of response of human adenocarcinoma C85 cells to clinically relevant MTX doses.
Methods: CytoTox-One Homogeneous Membrane Integrity assay (Promega) was performed on C85 cells exposed to MTX concentrations ranging between 10^{-9} – 10^{-5} M. Growth arrest was followed by 5-bromo-2-deoxyuridine (BrdU) incorporation assay in cells either exposed to 1 μM MTX or exposed to 1 μM MTX and allowed to recover after treatment. Cell cycle distribution of cells exposed to 1 μM MTX for 48 h and allowed to recover for subsequent 96 h, was analyzed using BD FACSCalibur flow cytometer and CellQuest software. Western blot analysis was employed to determine $p21^{Waf1/Cip1}$, $p16^{INK4a}$ and dihydrofolate reductase (DHFR) levels in C85 cells treated as in the case of cell cycle analysis. Senescence-associated β-galactosidase (SA-β-Gal) activity was visualized microscopically in C85 cells allowed to recover for 96 h after 48 h exposure to 1 μM MTX, using 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside as a chromogenic substrate.
Results: LDH release from C85 cells exposed to MTX showed the lowest rate for cells treated with 10 μM MTX for 48 h and allowed to recover for 72 h, with 14-fold reduction *vs.* cells grown under control conditions (each experiment performed in triplicate). In cells allowed to recover for 48 or 96 h after 48 h exposure to 1 μM MTX, BrdU incorporation was reduced by 80% (average from three experiments). Fraction of polyploid cells remained constant at 10% level in (i) control cells, (ii) cells exposed to 1 μM MTX for 48 h and (iii) cells exposed to 1 μM MTX for 48 h and allowed to recover for 96 h. In the course of MTX exposure and recovery, $p16^{INK4a}$ expression was reduced, $p21^{Waf1/Cip1}$ induced but DHFR level was maintained.
Conclusions: MTX-induced senescence in C85 cells is manifested by a permanent G1 phase growth arrest, SA-β-Gal expression and induction of $p21^{Waf1/Cip1}$ expression. It is not accompanied by any increase in polyploidy and, despite apparent growth arrest, no change of DHFR level is observed.

The Effect of 4Hz (30dB) Infrasound on Heart Muscle Contractility

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Background: Previously was shown that Infrasound (IS) at 4 Hz (30dB) frequency had modulating effect on heart muscle contractility. The molecular mechanism underlying this influence is not clear yet. The purpose of the present work is to study the molecular mechanism of infrasound. We have studied the effect of 4Hz (30dB) infrasound on heart muscle contractility, and Na/K pomp activity. We also measured the intracellular concentration of Ca^{2+} ions by ^{45}Ca, and the content of intracellular cyclic nucleotides (cAMP, cGMP).
Methods: Removed snail (Helix pomatia) hearts were cannulated and suspended in bath with physiological solution (PS). PS was exposed to 4Hz (30dB) IS for 30 minutes. ^{45}Ca uptake by muscles incubated in normal and IS-treated PS was measured by ^{45}Ca isotope. ^{45}Ca-uptake and intracellular cyclic nucleotides contents (cAMP and cGMP) were measured by Wallac 1450 liquid scintillation counter.
Results: IS at 4 Hz (30dB) frequency increased the amplitude and frequency of heart muscle contractility as well as the intracellular Ca^{2+} ions concentration. By measuring the content of intracellular cyclic nucleotides (cAMP, cGMP) we observed that by the increase of intracellular Ca^{2+} concentration caused the increase of cGMP content and decrease of cAMP contents in the cells.

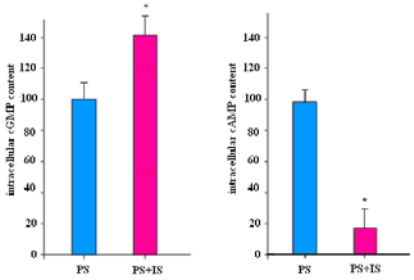

Figure
The effects of 4Hz (30dB) IS treated PS on the intracellular contents of cGMP and cAMP of heart muscle.

Conclusions: It is suggested that IS at 4Hz frequency and (30dB) intensity – induced transient relaxation of heart muscles caused the activation of Na/K pump in result of the increase of cGMP-dependent Ca efflux.

Genetic immunization: comparison of water-in-oil liposome-based delivery of cDNAs with *in vivo* electroporation.

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With the current shift from genomics to proteomics, monoclonal antibodies (mAbs) are expected to become a $50 billion market by the year 2012. However, making truly useful monoclonal antibodies that target native forms of proteins has been hampered by challenges in protein production and purification. Membrane proteins, for instance, are difficult to purify yet are among the best candidates for drug development because 50% of the current drugs on the market work via membrane proteins. We have developed improved methods that bypass the need for purification of proteins for immunization or for the screening of hybridoma supernatants. We have used two different DNA delivery methods into mice: (I) "in vivo electroporation", using CUY21SC from Protech International, Inc., and (II) water-in-oil liposome-based platform from ImmunoVaccine Technologies. The genes of interest (such as Human L1) were placed downstream of the immediate early promoter/enhancer sequences of cytomegalovirus intron A immediate-early (IE) gene. Mice received 100 µg of plasmid by intra-dermal injection. Sera and positive clones were screened using an improved FLISA screen on fixed/permeablized/blocked Cos-7 cells transfected with the genes of interest plated in 96-well plates and read by LI-COR Odyssey. Fusions were performed using Stemcell Technologies fusion protocol modified to take advantage of a stereo Video microscope for picking clones. While both immunization protocols resulted in strong protein expression/immune responses, in vivo electroporation resulted in the expression of protein mainly in the injection site reaction while the water-in-oil liposome emulsion directed the expression of the delivered gene into the lymph nodes. Collectively, these methods increase the breath, magnitude, and durability of immune responses to native antigens and importantly, eliminate the need to produce and purify large quantities of protein for immunization, dramatically reducing monoclonal antibody production costs. Incidentally, our data suggest that described methods may be used for nucleotide delivery in general such siRNA, RNA and DNA.

Acknowledgements
Protech International, Inc. for providing the electroporator and electrodes. ImmunoVaccine Technologies Inc. for providing the water-in-oil liposome platform. Development costs supported by the Wallace H. Coulter Center for Translational Research and the University of Miami Neuroscience Center.

Cyclotides, ultrastable peptide frameworks for magic bullet drug delivery

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Background: Cyclotides are plant derived mini-proteins with compact folded structures and exceptional stability. Their stability derives from a head-to-tail cyclized backbone coupled with a cystine knot arrangement of three-conserved disulfide bonds. Taking advantage of this stable framework we developed novel VEGF-A antagonists by grafting a peptide epitope involved in VEGF-A antagonism onto the stable cyclotide framework. Antagonists of this kind have potential therapeutic applications in diseases where angiogenesis is an important component of disease progression, including cancer and rheumatoid arthritis.
Methods: This study involved the use of solid phase peptide synthesis to produce several analogues of the prototypic cyclotide kalata B1 with a poly-arginine epitope that specifically inhibits the interaction of VEGF with the KDR receptor, grafted into the backbone. The peptides were cyclized and oxidized and their structures analzyed using NMR spectroscopy. Their biolgoical activity was assessed in a cell based VEGF-A antagonism assay and their in vitro stability determined in human serum.
Results: The grafted peptides were successfully cyclized and oxidized and maintained the native cyclic cystine knot fold. Furthermore the grafted peptides were more stable in human serum than the linear poly R epitope. The most active analogue was a graft into loop 3, which showed low micromolar activity in the VEGF-A antagonism assay. Calculation of the three-dimensional structures suggested that disorder in the poly R region might allow adaptation to the receptor site and facilitate biological activity.
Conclusions: We confirmed that a range of grafted cyclotide analogues can be synthesized and form a cystine knot motif with stability in human serum. The large number of known cyclotide sequences, which can be thought of as nature's combinatorial library of cyclotides, helped to direct the design process for the grafted analogues. Biological activity was maintained in one of the grafted analogues, validating the potential of this framework in drug design. Given that the epitope grafted into the scaffold differs significantly from the native sequence, it appears likely that the cyclotide framework can accommodate a wide range of epitopes, broadening its scope in drug design.

Personalized Prevention of Colorectal Neoplasia: High Magnesium and Low Calcium among People with a Functional Polymorphism in the Magnesium Transporter

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Background Growing evidence from studies conducted in Western societies has linked low intake of magnesium to insulin resistance and systemic inflammation and, thus, risk of diseases common in Western countries, such as colorectal cancer, type II diabetes and coronary heart disease. Migration studies found that after moving to Western societies, the incidence of the aforementioned diseases in East Asian immigrants, a population with traditionally low risks, approached that for Caucasians. We found that mean intake of magnesium in the US population is, however, not different or even slightly higher than that in East Asia. Instead, the ratio of calcium to magnesium intake is much higher in the US population (2.8)than in East Asia (1.6). Transient receptor potential melastatin 7 (TRPM7) is a newly found gene essential to magnesium absorption and homeostasis.
Methods: To test whether the association of colorectal polyps with intake of calcium and/or magnesium and Thr1482Ile polymorphism in the TRPM7 gene is modified by the calcium/magnesium intake ratio. Included in the study were a total of 688 adenoma cases, 210 hyperplastic polyp cases, and 1,306 polyp-free controls from The Tennessee Colorectal Polyp Study (TCPS).
Results We found total magnesium consumption was linked to a significantly reduced risk of colorectal adenoma, particularly for those with a low calcium/magnesium ratio. An inverse association trend was found for hyperplastic polyps. Further, we found the common Thr1482Ile polymorphism was associated with an elevated risk of both adenomatous and hyperplastic polyps. Moreover, this polymorphism significantly interacted with the calcium/magnesium intake ratio in relation to both adenomatous and hyperplastic polyps. People who carry at least one 1482Ile allele, and if they consumed diets with a high calcium/magnesium ratio, were particularly at an elevated risk of adenoma (odds ratio (OR) =1.60, 95% confidence interval (CI) =1.12-2.29) and hyperplastic polyps (OR=1.85, 95%CI=1.09-3.14) vs. those who do not carry the polymorphism. We will also update novel unpublished findings in the presentation.
Conclusions These findings, if confirmed, may provide a new avenue for the personalized prevention of magnesium deficiency and, thus, colorectal cancer.

Insulin Suppresses High Mobility Group-B1 (HMG-B1) Protein and Toll Like Receptor Expression: Further Evidence of its Anti-Inflammatory Effect

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We have previously shown that insulin at low doses exerts a potent and rapid anti-inflammatory effect through the suppression of pro-inflammatory transcription factors, NFkB and Egr-1. In addition, insulin suppresses plasma CRP, SAA and PAI-1 concentrations and may reduce the size of the infarct in patients with acute myocardial infarction. Our recent work has demonstrated that the expression of toll like receptors, TLR-2 which recognizes gram positive bacterial lipopeptides, and, TLR-4 which is the specific receptor for endotoxin, is also suppressed by insulin infused at a low dose (2U/h). Insulin also suppresses the activity of PU.1, the transcription factor which regulates TLR expression. TLRs 4 and 2 mediate inflammation induced by endotoxin and Gram positive bacteria while they are also known to mediate diet induced obesity related insulin resistance and injury related to ischemia/reperfusion. Thus, insulin may have potentially important therapeutic effects in endotoxemia, bacteremia and septicemia. Our most recent work shows that a low dose infusion of insulin (2U/h) leads to a reduction in the expression of HMG-B1, an intranuclear chromatin related protein which is able to open the nucleosomes such that gene promoters are can be bound by RNA polymerase II and pro-inflammatory transcription factors leading to the transcription of inflammatory genes. Since endotoxin induced inflammation is mediated by TLR-4 (receptor for endotoxin) and NFkB whose action is amplified by HMG-B1, these actions of insulin are appropriate for controlling endotoxin induced inflammation. Our most recent work also shows that the intake of cream and a high fat high carbohydrate meal results in an increase in plasma endotoxin concentrations and may therefore contribute to post prandial oxidative stress and inflammation. Thus, the natural post prandial increase in insulin may neutralize the effects of macronutrient related increase in endotoxin. Corticosteroids are the most commonly used anti-inflammatory agents and therefore we examined the effect of hydrocortisone on these indices. It is of interest that a high dose of hydrocortisone does not affect the expression of TLR-4 while it paradoxically increases the expression of HMG-B1. Clearly, therefore, insulin is potentially the ideal agent for controlling endotoxin induced inflammation. These actions may have contributed to the success of previously reported studies using insulin in the setting of intensive care. More prospective controlled studies are required for insulin alone and in combination with corticosteroids. Furthermore, the cardioprotective effect of insulin related to its vasodilatory, anti-inflammatory, anti-platelet, anti-thrombotic and anti-apoptotic actions is currently being tested by us in 600 patients with acute anterior ST elevation myocardial infarction in a prospective study measuring the size of the infarct MRI to demonstrate a reduction in the size of the infarct. Our work has clearly opened up several areas of inflammation and cardioprotection which require careful investigation for the potential novel beneficial effects of insulin.

Bis(2-aminoimidazolinium)diphenyl Compounds as DNA Minor Groove Binders with *in Vivo* Antitrypanosomal and Antimalarial Activity: the cation is important.

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Background: A pragmatic approach to the discovery of new drugs for neglected diseases is the "recycling" of available compounds. We have successfully applied this strategy during the last years with the (re)discovery of an attractive class of compounds (i.e., 2-aminoimidazolinium derivatives) showing excellent *in vivo* activity against *T. brucei rhodesiense* and *P. falciparum*, the ethiological agents of rhodesiense sleeping sickness and severe malaria, respectively.
Methods: Based on their structural similarity with known antitrypanosomal and antimalarial agents, several series of dicationic compounds as well as their monocationic and neutral analogues were screened *in vitro* against *T. b. rhodesiense*, *P. falciparum*, and rat skeletal myoblast L6-cells as control for cytotoxicity. The compounds showing the highest activity and acceptable selectivity were assayed *in vivo* in models of acute and chronic *T. brucei* infections (STIB900 and GVR35 strains, respectively), and murine malaria (*P. berghei*). Their interaction with the DNA minor groove was also measured by thermal melting curves (ΔT_m) and SPR experiments on AT sequence DNA polymers.
Results: Several dicationic leads with nM *in vitro* activity and excellent selectivity against *T. b. rhodesiense* and *P. falciparum* were identified. A number of compounds cured 100% of the mice infected with *T. b. rhodesiense* and 4 compounds reduced the parasitemia in mice infected with *P. berghei*. A correlation between DNA binding affinity and trypanocidal activity was observed, indicating that DNA binding may be part of their mechanism of action. Most importantly, we found that the 2-aminoimidazoline cation afforded molecules with superior safety profile compared with its guanidine counterpart.
Conclusion: 1) The rational screening of in-house libraries of compounds is a validated approach to find new drug leads for neglected diseases. 2) Bis(2-aminoimidazoline) derivatives represent a very promising class of DNA minor groove binding agents that have already demonstrated their antiprotozoal potential *in vivo*.

Dentistry and Antibiotic Resistance: The Need to Set Guidelines and Improve Prescribing Practices.

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Background: In the last decade, there has been an increasing interest in investigating antibiotic use by dentists. Aims: This study aims to evaluate 1) The degree of knowledge that the dental profession has, concerning therapeutic uses of antibiotics. Practices and knowledge of dentists worldwide will be assessed. 2) Deficits in our knowledge about antibiotics, and controversies about the correct use of antibiotics, will also be highlighted.
Methods: A literature review was performed in Pubmed during May and June-2008 using the keywords: antibiotics, dentistry, prescription. All articles that were written in English and which investigated dentists' knowledge about antibiotics in different countries were reviewed.
Results: A large gap in knowledge was found between dentists practicing in Europe and North America and dentists who work in developing countries. Defects in knowledge about antibiotics were mainly in the fields of: indications for the use of therapeutic antibiotics, duration of antibiotic therapy, and alternatives to penicillin in penicillin-allergic patients.
Conclusions: More appropriate methods for dental treatment should be employed to reduce unnecessaary antibiotics consumption. These methods may include appropriate dental treatment, analgesic therapy, and education of the patient. The medical and dental schools should improve their curriculum of microbiology. Efforts should be united to counteract this problem by encouraging collaboration of experts and scientists as well as decision-makers worldwide.

The Role of PPARγ Agonist in Alzheimer's disease.

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Abstract--Alzheimer's disease is the most common cause of dementia. The increasing prevalence of the disease presents a challenge to the treating neurologist. Presently the drugs used for Alzheimer's disease produce only symptomatic improvement. Currently various therapeutic strategies are under development for the treatment and prevention of Alzheimer's disease, like γ secretase inhibitors, immunotherapy with anti Aβ antibodies,Aβ immunization, metal protein attenuation compounds, and peroxysomal proliferators activated receptors γ agonist,NSAIDS. Alzheimer's disease has a marked inflammatory component. It is proposed that anti inflammatory therapies may be of value in Alzheimer's disease. PPARS are members of nuclear hormone receptor super families that are activated by lipids in regulating inflammatory responses. PPARγ has been investigated in animal model for its role due to its anti inflammatory action in various C.N.S. diseases, having an inflammatory component. PPAR γ agonists have been demonstrated to suppress amyloid β mediated activation of microglia in vitro and to prevent cortical and hippocampal neuronal death. Various studies have shown that PPAR γ agonists may be of value in treatment of Alzheimer's disease in animal models. The details of PPARγ agonists as a therapeutic target for the treatment of Alzheimer's disease will be discussed.

Abbreviations.-PPARγ (Peroxysomal Proliferators Receptor Activator Gamma), Aβ (Amyloid beta), NSAIDS (Nonsteroidal Anti-Inflammatory Drugs), C.N.S. (Central Nervous System).

Experimental studies on antimicrobial potentialities of antipsychotics and antiinflammatory drugs with special reference to their action on Mycobacterium tuberculosis

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Background: Excessive and inadvertent use of antibiotics and antibacterial chemotherapeutics has caused a significant increase in the occurrence of drug resistant pathogens including M. tuberculosis. Systematic search among various pharmacological categories of drugs have revealed that many of them possess potent antimicrobial property.
Methods: The antipsychotics and antiinflammatory drugs were tested in vitro against 300 to 500 bacteria including two cag positive H. pylori and 45 mycobacteria. The MIC was determined by agar dilution technique following NCCLS guidelines along with detection of bactericidal/bacteriostatic action. Intensive in vivo experiments were carried out in mice employing S. enteritidis var typhimurium and M. tuberculosis H37Rv. Protection against V. cholerae was established in rabbit ileal loop model.
Results: Antipsychotic drugs fluphenazine, flupenthixol, trifluoperazine and antiinflammatory agent diclofenac sodium possess remarkable antimicrobial effect against clinical isolates of Gram positive and Gram negative types. Diclofenac was found to inhibit synthesis of bacterial DNA. These compounds when injected intraperitoneally significantly protected Swiss white mice from the lethality of Salmonella infection. When tested in vitro these drugs could inhibit mycobacteria at 10-25?g/ml level. Diclofenac sodium when injected at 10 ?g/gm body weight resulted in statistically significant survival ($p<0.01$ according to ?2 test) of mice challenged with 50MLD of M. tuberculosis H37Rv. Flupenthixol provided visible inhibition in the ballooning of rabbit ileal loop infected with V. cholerae 569B.
Conclusion: Definite detection of antibacterial particularly antitubercular functions in antipsychotics and antiinflammatory drugs may prove to have a profound impact in the treatment of various infections including tuberculosis. In course of time it will be possible to create a new generation of potential antimicrobics by suitable structural modification of these agents. Thus development of novel chemotherapeutic compounds would retain a paramount legacy in the history of therapy of infections as envisaged by Paul Ehrlich.

Targeting of blood vessels by small ApoE-like peptides

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Background: The prerequisite for the successful application of all biological agents is the access to the site of action. Drug application to the brain is a particular challenge. One approach to overcome the blood brain barrier (BBB)-related limitations is the development of drug carriers and their modification with peptides to take advantage of physiology-based strategies.
We introduced a modified apoE-derived sequence, A2 $(LRKLRKRLLR)_2$ as target recognizing and uptake mediating compound. The peptide exhibits characteristics of cell penetrating peptides (CPPs), comprises specific binding sites for the low density lipoprotein receptor (LDLr) and binds non-specifically to cell-surface heparan sulfate proteoglycans (HSPGs). These properties provide the potential for the activation of different cellular uptake modes. Introduction of two palmitoyl chains conferred detergent-like properties upon the cationic sequence. This lipopeptide allowed rapid and easy formation of different particulate systems.
Methods: Spectroscopic and calorimetric methods were used to characterise the particles and confocal laser scanning microscopy (CLSM) and fluorescence assisted cell sorting (FACS) were used to monitor cellular uptake.
Results: The peptide mediated efficient non-selective cellular uptake of liposomes into different cell lines such as endothelial cells of brain capillaries and large vessels. The uptake mode into capillary endothelial cells is endocytotic, but neither clathrin nor caveolae mediated. The LDLr does not play a role. Cell surface HSPGs are involved in the uptake process, providing an explanation for non-specificity and leading to the suggestion that electrostatic interactions between the carrier and cell membrane initiate macropinocytosis. In contrast, the cellular uptake of micelles is cell specific. P2A2 micelles are efficiently internalized into capillary endothelial cells whereas the uptake into endothelial cells of large vessels is low. The observations imply that on the various cell species different transport routes are activated and the properties of the particulate carrier, such as the size, surface density of the vector peptide, and peptide conformation influence the process.
Conclusion: Our studies lead to the conclusion that small micellar particulate structures with a high surface density of the cationic apoE vector peptide are highly favourable for the uptake into brain capillary endothelial cells.

Polymyxin B: how this Magic Bullet kills Gram-negative Bacteria?

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Background: Polycationic antimicrobial peptides are an important component of the innate defence of all species of life. These compounds are active against a broad range of bacterial strains, including antibiotic resistant isolates, and are synergistic with conventional antibiotics. The therapeutic use of a cationic antibiotic polymyxin B (PMB) was abandoned for a long time due to its undesirable side effects. However, the spread of resistance to currently used antibiotics has forced the reevaluation of PMB for clinical use. Using different substrates and inhibitors of energy metabolism we obtained information on the mechanism of PMB interaction with bacterial outer membrane (OM) as well as with the plasma membrane (PM).
Methods: An electrochemical monitoring of K^+, Ca^{2+}, H^+, tetraphenylphosphonium (TPP^+), and phenyldicarbaundecaborane (PCB^-) ion fluxes across envelopes of *E. coli* and *Pseudoalteromonas* spp. cells was performed. In parallel, the cell binding of fluorescent compound dansylpolymyxin, OD of bacterial suspensions, ATP content of cells, and bactericidal activity of PMB were studied.
Results: Using different conditions of cell incubation, the OM permeabilizing activity of PMB was dissected from the PM depolarizing effects. These two stages were easily distinguishable in the presence of high concentrations of divalent cations and can be separated in time by 1-5 min interval. PMB-induced pores in bacterial envelope were registered, but the pore formation and depolarization of the PM were not obligatory for the dissipation of cell K^+ gradient or the bactericidal action of this antibiotic. At conditions of increased ionic strength the dependence of membranotropic activity of PMB on metabolic state of the cells was discovered. Energization of the cells by glucose stimulated the binding of PMB to bacteria and the depolarizing activity of this antibiotic. Membranotropic effects of PMB were considerably stronger when all amount of the drug was added to the cell suspension at one stroke.
Conclusions: 1. At low concentrations PMB compromises the barrier of the OM, while at higher concentrations it also depolarizes the PM by forming ion-permeable pores. 2. High ionic strength prevents the self-promoted entry of PMB into bacterial cells, though the ability to bind to the OM surface is not affected. 3. Suppression of energy metabolism of bacteria makes them more resistant to PMB.

Molecular Mechanism of Action and Cellular Consequences of the DNA Minor Groove Alkylating Agent S23906-1.

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Background: Derived for the plant alkaloid acronycine, the drug candidate S23906-1 was synthetised on the basis of the stabilization of the natural reactive epoxide derivative of acronycine and addition a linearized phenyl group to increase its propensity to alkylate DNA. From a series of derivatives, S23906-1 was selected to enter in phase 1 clinical trial over its potent cytotoxic effects and its high antitumor potency on a wide variety of pre-clinical models.
Methods: The precise mechanism of action of S23906-1 over DNA was assessed using various biophysical, molecular and cellular approaches among which mass spectrometry, HPLC, EMSA, nuclease S1 cleavage, genomic DNA fluorescence studies, cytotoxicity measurements and transfection analysis.
Results: At the molecular level, S23906-1 alkylates guanine at the N2 position as does ET-743 (Trabectedin, Yondelis ™). By contrast to ET-743, S23906-1 does not stabilize the double helix of the DNA but induces a local opening of the DNA. At the cellular level, S23906-1 induces apoptosis in manner directly dependent on its DNA alkylation potency in correlation with double stand break formation in cells. In order to evaluate the implication of the local destabilization of the DNA helix on cellular toxicity, we identified, using a proteomic approach, glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) as a protein specifically interacting with S23906-1 DNA adduct. GAPDH is a multifunctional protein implicated in glycolysis, DNA repair and apoptosis processes but also interacts with both double- (dsDNA) and single-stranded DNA (ssDNA). Using EMSA, no binding of GAPDH on ET743/DNA adduct was evidenced. The cellular consequences of GAPDH interaction with S23906-1/DNA adducts are evaluated using siRNA and transfection approaches.
Conclusions: S23906-1 is a potent DNA minor groove alkylating agent presenting particular molecular and cellular mechanisms of action contrasting with that of other DNA minor groove alkylating agents such as ET-743. Evidencing the role of specific DNA adduct recognition by cellular proteins would help understanding the link between the primary DNA modification and the resulting cell death process.

New neutralizing monoclonal antibodies from HIV-1 subtype C and CRF02_AG infected people.

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Background: The aim is to develop prophylactic human immunodeficiency virus vaccines targetting subtype C and circulating recombinant form CRF02_AG which are currently most prevalent. The recombinant prime, peptide boost immunization strategy has protected rhesus macaques against infection with a neutralization sensitive SHIV which, however, was constructed from a subtype B isolate albeit a variant of the immunogens. Protection correlated with activity in extended incubation phase neutralization assays against virus prepared in primary human cells. To develop this immunization strategy further, combinations of immunogens which can induce antibodies able to cross-neutralize fully heterologous isolates have to be identified. First, monoclonal antibodies with these properties need to be isolated. They can then be used to select recombinant glycoproteins and construct peptides.
Methods: The subtypes of HIV-1 from infected patients attending the clinic in Antwerp were determined. Plasma from individuals infected with subtype C or CRF02_AG was mixed with dilutions of primary virus from a panel of HIV-1 isolates and incubated for 24 hours. Residual infectious virus was quantified following exposure of this mixture to phytohaemagglutinin transformed human peripheral blood mononuclear cells for one hour. The presence of infectious virus was determined by HIV-antigen ELISA of supernatants after 14 days' culture. Reductions in infectious titre were calculated and expressed as neutralization indices. Memory B lymphocytes from individuals with cross-neutralizing antibodies were isolated, stimulated to divide and immortalized by Epstein-Barr virus. Dividing cultures were initially screened for antibodies binding to recombinant HIV-1 envelope gp140. Neutralizing activity of the monoclonal antibodies was subsequently determined in a standardized pseudovirus HOS.CD4-CCR5 based cell assay.
Results: Patients with plasma showing subtype-associated cross-neutralization were identified. So far, two antibodies with broad cross-neutralizing activity and a potency comparable to currently available human monoclonals have been isolated. Other antibodies have a more restricted range while recognising a variety of known epitopes on the external envelope glycoproteins although a subgroup bind to potentially novel neutralization epitopes.
Conclusions: New cross-neutralizing antibodies are available which can identify immunogens for use in recombinant prime, peptide boost immunization strategies against HIV-1 subtype C and CRF02_AG isolates.

Effect of antineoplastic agents on the surface properties of bacterial cells

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Background: The study of the effects of antineoplastic agents on bacteria is important to understand post-chemotherapy infections, generally only ascribed to immune suppression. Aims: 1) To evaluate the effect of drugs, such as mitomycin C and cyclophosphamide, on the viability and surface properties of bacteria. 2) To assess if the cells that survive exposure to these agents are also able to grow with antibiotics present.
Methods: Exponentially growing cells of *Staphilococcus aureus*, *Rhodococcus erythropolis*, *Mycobacterium aurum* and *Escherichia coli* were placed in medium containing 100, 50, 25, 12.5, 6.3, 3.1, 1.6 and 0.8 μg/mL of mitomycin C (MC) or cyclophosphamide (CPP). The fatty acid composition of the cellular membranes was analysed by gas chromatography. Cell viability and membrane potential were determined by fluorescence microscopy and image analysis, using fluorescent dyes. Culturable cells were determined by the spread plate technique. Cell hydrophobicity was measured by the "microbial adhesion to hydrocarbon" test. The size of cell clusters and biofilm formation were determined by microscopy and image analysis.
Results: Both MC and CPP affected the fatty acid composition of the cellular membrane of all strains. A dose dependent increase in the degree of saturation of fatty acids occurred after exposure to MC. Both agents caused a dose dependent decrease in cell viability, part of the population presenting depolarised membranes. Cells exposed to MC produced biosurfactants, decreasing the medium surface tension and *R. erythropolis* cells produced significant amounts of exopolymeric substances on agar plates after 24h exposure to both agents. Cell clustering was promoted by increasing concentrations of these drugs. Cells that were able to grow after 24h exposure to each of the antineoplastic compounds, were also able to grow in the presence of 25 μg/mL of chloramphenicol, suggesting cross-resistance between them.
Conclusions: 1) Bacterial cells are able to survive relatively high concentrations of both MC and CPP. 2) These cells can also grow in the presence of chloramphenicol. 3) Post-chemotherapy infections could be promoted by an adapted bacterial population after antineoplastic exposure.

The High Cytotoxicity of Cisplatin Nanocapsules in Ovarian Carcinoma Cells Depends on Uptake by Caveolae-Mediated Endocytosis

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Background: Cisplatin is one of the most widely used agents in the treatment of solid tumors. However, its clinical use is limited by toxic side effects and by the occurrence of resistant tumor cell sub-populations. We have developed a novel method for the efficient encapsulation of cisplatin in a lipid formulation that may reduce the side effects and increase the therapeutic efficacy of cisplatin in the clinic (Burger *et al.*, Nat. Med. 8, 2002). The method is unique in that it generates nanocapsules, nanoprecipitates of cisplatin covered by a single lipid bilayer. The nanocapsules exhibit an *in vitro* cytotoxicity up to 100-fold higher than the free drug toward some but not all human ovarian carcinoma cells. Here we report on the mechanism underlying the cell line dependence of the increased cytotoxicity.
Methods: Cellular platinum accumulation and platinum-DNA adduct formation were analyzed by non-flame atomic absorption spectroscopy. The interaction of fluorescently labeled cisplatin nanocapsules with ovarian tumor cells was investigated by confocal fluorescence microscopy. Endocytic pathways were down-regulated using siRNAs.
Results: The increased cytotoxicity of cisplatin nanocapsules was shown to result from enhanced cellular uptake of encapsulated cisplatin as compared to the free drug, leading to increased formation of platinum-DNA adducts. The origin of the increased accumulation of cisplatin from nanocapsules in the cells was investigated by confocal fluorescence microscopy using nanocapsules containing a fluorescent cisplatin derivative. The results showed that intact nanocapsules are taken up by an energy-dependent mechanism. Co-localization of the fluorescently labeled nanocapsules with markers of early and late endosomes indicated uptake via endocytosis. Transfection of siRNAs against clathrin heavy chain and caveolin-1 in cell lines that differ in sensitivity to cisplatin nanocapsules, revealed that the increased cytotoxicity only occurs after caveolin-1 mediated endocytosis.
Conclusion: The high cytotoxicity of cisplatin nanocapsules in human ovarian carcinoma cells strictly depends on uptake by caveolin-1 mediated endocytosis.

***Pneumocystis jirovecii* Dihydropteroate Synthase Gene Mutations and Sulfa Resistance**

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Background: Sulfa drugs, trimethoprim-sulfamethoxazole and dapsone, are mainstays for prophylaxis and treatment of *Pneumocystis* pneumonia (PcP), a life-threatening disease in immunosuppressed patients. The inability to culture *Pneumocystis* has led to develop molecular techniques based on identification of punctual mutations on the Dihidropteroate Synthase gene (DHPS) that cause sulfa resistance in other microorganisms. A key issue is whether the emergence of DHPS mutations is result of transmission between patients or arises from selection by the pressure of sulfa drugs, two possibilities are not mutually exclusive. The role of *Pneumocystis* colonized subjects in transmission of DHPS mutations still unknown. The aim is to provide epidemiological data of *P. jirovecii* DHPS mutations among PcP patients and immunocompetent colonized subjects.
Methods: The study included 47 PcP patients and 75 *Pneumocystis* colonized subjects during 2001-2007 identified by nested PCR at mtLSUrRNA gene. DHPS mutations were studied by Restriction Fragment Length Polymorphism using *Acc I* and *Hae III* at nucleotide positions 165 and 171 respectively.
Results: The analysis showed a 19.7% prevalence of DHPS gene mutations in the overall population. , All possible polymorphisms described were identified. There were not difference between the frequency of DHPS mutations in PcP patients and colonized subjects (23.4% *vs* 17.3 %; p = 0,75). A trend towards decreased frequency was observed during this period (31.3% of DHPS-mutations during 2001 to 11.6% at 2007).
Conclusions: Similar DHPS pattern was observed in PcP patients and immunocompetent colonized subjects suggests that both group could share a common transmission cycles of mutated strains, and arise question about the role that colonized subjects could represent as reservoir for DHPS mutations with ability to transmit them to immunocompromised hosts susceptible to PCP.

A Simple Microwave-assisted Synthesis of Sulfonamides directly from Sulfonic Acids

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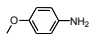
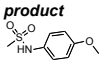
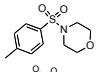
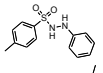
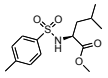
Background: Sulfonamides are an important class of pharmaceutical compounds with a wide spectrum of biological activities. Sulfonamides drugs have broad applications in many areas of clinical medicine, as excellent, diuretics, anticonvulsants, hypoglycemics HIV protease inhibitors, carbonic anhydrase, and caspase inhibitors and in particular as antibacterials.
Methods: We wish to report here an easy and convenient technique for the preparation of sulfonamides directly from sulfonic acid or its sodium salt, improved by microwave irradiation. The method consists of the addition of 1 equiv. of TCT to a mixture of 1 equiv. of sulfonic acid and 1 equiv. of triethylamine in acetone. The reaction was carried out under microwave irradiation in a sealed tube (10-mL pressure-rated reaction vial) in a self-tuning single mode irradiating synthetizer, operating at 80 °C for 20 min. After cooling, the precipitate formed is filtered off on Celite and the solution is added with 1.2 equiv. of $NaOH_{aq}$, THF and an amine. The reaction mixture is newly exposed to microwave irradiation for 10 min at 50 °C in a sealed tube and then is filtered on Celite to eliminate the formed salts, diluted with DCM and washed with water, aqueous Na_2CO_3, diluted HCl, and brine. The target product is obtained in pure form and in practically quantitative yield, just by concentration of the DCM extracts at reduced pressure.
Results: A selection of sulfamides were synthesized from an array of sulfonic acids and the yields were satisfactory in all cases. The methodology is proficient and successful with aromatic and aliphatic sulfonic acids. The reaction is not limited to primary and secondary amines, but works well with hydrazines (entry 3) and amino acid derivatives (entry 4). Also anilines are applicable in the reaction (entry 1).

entry	*sulfonic acid*	*amine*	*product*	*yield%*
1	H_3C-SO_3H			90
2				95
3	"			92
4	"			80

Conclusions: The methodology described represents a convenient,handly and high yielding synthesis of sulfonamides even in large scale, as it uses mild reaction conditions and cheap and commercially available reagents.

All abstracts are listed in alphabetical order of the presenting author.

In Vitro Fungicidal Properties of the Plant Saponin, CAY-1, with (1) Two CAY-1 Structurally Related Saponins and (2) Synergism with Silver.

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Background: CAY-1, 1081 and 919 are structurally similar saponins in *Capsicum frutescens* fruit. CAY-1 is fungicidal or inhibitory for many fungi including *Aspergillus, Fusarium, Microsporum* and *Trichophyton* species. In contrast, 1081 and 919 are weakly antifungal and inactive, respectively. Historically, silver has been used as an antimicrobial. This study determined the **(1)** fungicidal properties of CAY-1 alone and combined with 1081 and 919 and **(2a)** fungicidal properties of silver and CAY-1 both alone and mixed and **(2b)** synergy of silver and CAY-1.
Methods: (1) The fungicidal activity of CAY-1, 1081 and 919 mixed in ratios of 8:1:1, 6:2:2 and 4:3:3 (found in *Capsicum*), were compared to equal weights of CAY-1 alone. Nongerminated (NG) and germinating (G) conidia of *A. flavus, A. niger, A. fumigatus, F. solani, F. oxysporum* and *F. moniliforme* were tested to determine activity against such conidial types. Separate bioassays (3) were performed (n=12) for each species and conidial type with results analyzed statistically using SigmaStat. **(2)** This fungicidal protocol and Minimum Inhibitory Concentration (MIC) bioassays were performed with dissolved silver and CAY-1 both alone and mixed.
Results: (1) Overall, mixture 4:3:3 was the most active. For *A. flavus* and *A. niger* G conidia, CAY-1 alone was significantly ($p < 0.001$) lethal at 5.3 and 6.6 µg/ml, respectively, and for mixture ratio 4:3:3 at 5.0 and 6.1 µg/ml, respectively. Significant lethality for *F. solani* G conidia was achieved with CAY-1 alone and 4:3:3 mixture at 19.8 and 6.6 µg/ml, respectively. CAY-1 was inactive against *F. moniliforme* but mixture 4:3:3 was lethal at 3.0 µg/ml. **(2)** CAY-1 significantly reduced the G conidial viability of *A. flavus, A. niger* and *F. solani* at 1.3, 0.64 and 12.4 µg/ml, respectively. Silver (0.64-79.4 µg/ml) was significantly lethal for all fungal G conidia and the NG conidia of *F. oxysporum* and *F. solani.* Combined, silver and CAY-1 were significantly lethal for all fungi at concentrations inactive when tested separately. MIC data showed combined CAY-1 and silver had an additive synergistic effect.
Conclusions: Results suggest that the amount of CAY-1 needed for significant antifungal activity is reduced up to 60% by addition of inactive levels of related saponins or dissolved silver.

Validation of a RP-HPLC Method with Fluorescence Detection for the Bioequivalence Study of Norfloxacin in Plasma Samples

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A robust method for the determination of norfloxacin in human plasma using reversed-phase high performance liquid chromatography (RP-HPLC) with fluorescence detection has been developed. The method involved precipitation of plasma protein with acetonitrile using ciprofloxacin as internal standard (IS). Chromatographic separations were performed on a Synergi MAX-RP 150mmx4.6mm, 4µ column with an elution system consisting of a mixture of phosphate buffer-acetonitrile (85:15, v/v). The calibration curve was linear in the range of 30 – 3500 ng/mL. The recoveries at concentrations of 90, 1400 and 2800 ng/mL were 103.5%, 100.2% and 100.2%, respectively. The quantification limit for norfloxacin was 30 ng/mL per 10 µL injection employing fluorescence detection with excitation and emission set at 300 and 450 nm, respectively. The method validation included examining the within-run and between-run precision and accuracy and ensuring that these were within accepted limits; in summary, the precision was <8.6% and accuracy ranged from 95.8%-104.1% for concentration from 90-2800 ng/mL. The precision and accuracy for the lowest calibration standard (30 ng/mL) was well within accepted limits for LOQ. The method was then applied in a bioequivalence study in healthy volunteers given 400 mg doses of reference and test formulations of norfloxacin in random order and including a 7-day washout phase.

The Pharmacokinetics and Pharmacodynamics of Miltefosine for Leishmaniasis.

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Background: Miltefosine is an alkylphosphocholine with activity against leishmaniasis. Pharmacokinetics and -dynamics have little been studied.
Methods: A review will be given, combining data from literature and our own data.
Results: The pharmacokinetics of miltefosine, administered orally to healthy subjects and patients with cutaneous leishmaniasis, is characterised by a rapid absorption and a very slow, two compartment, elimination proces with elimination half lives of 7 and 31 days. After four weeks of oral treatment, miltefosine remains detectable in plasma for up to 5 or 6 months. The effects of hepatic leishmaniasis on drug disposition have not been studied yet.
Toxicity of miltefosine is determined by dose related gastrointestinal adverse effects that are amenable by concurrent food intake and disappear soon after discontinuation of drug administration. The relation between miltefosine plasma concentrations and other adverse effects has not been fully investigated.
The pharmacodynamics of antileishmanial activity of miltefosine is poorly documented. In vitro data show different sensitivity of *Leishmania* species but it is difficult to extrapolate this to clinical efficacy. Efficacy is usually expressed as cure rates in clinical trials which may be higher than 95% for Indian visceral leishmaniasis but lower for other *Leishmania* infections. The few available data on parasite clearance indicate that this continues until after discontinuation of miltefosine administration. The pharmacokinetics and -dynamics of systemic treatment for cutaneous leishmaniasis and of topical treatment have not been studied. The little available data on eradication time, parasite sensitivity, host immunity, other pharmacodynamic determinants and the sparse data on drug interactions will be will be reviewed.
Conclusions: The pharmacokinetics and -dynamics of miltefosine need further study to design rational treatment regimens that address (selection of) resistant parasites, cutaneous leishmaniasis and combination with other antileishmanial agents and to study options for topical therapy.

From immunity theory to anti-infectious chemotherapy. Why awarding two Nobel prices in 1908 to Paul Ehrlich (from Germany) and Elie Metchnikoff (from France). What consequences for medical research?

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By 1908 Elie Metchnikoff and Paul Ehrlich jointly obtained the Nobel Prize of physiology and medicine for their work on immunity. Metchnikoff explained the phenomenon of the phagocytosis in observing the starfish larvae (1880), whereas Ehrlich developed the theory of fixing antigen/antibody. The end of the XIX^{e} century is dominated by the Pasteurian theories which were a great successes in the field of vaccination and serotherapy. First vaccines against variola, then the development of the diphtheria anti serotherapy had indeed nourished immense hope in the fight against the infectious diseases. Work on immunity thus were developed to understand the mechanisms brought into play and to generalize the immunological methods with the unit of the infectious diseases. Unfortunately, this very tempting ideal model is put in failure in the case of many infectious diseases and parasitic (tuberculosis, malaria, trypasonomiasis…). Ehrlich proposes another original model then. He considers that the antigen/antibody reaction is a chemical reaction. So, to fight against a microbe why not introduce into the host, an external chemical body which, like magic bullet neutralizes the cellular target? He carries out its first experiments with a dye: the methylene blue then with the trypanroth, whereas at the Pasteur institute of Paris Roux, Metchnikoff, Mesnil, Nicolle, and Laveran were also working on various dyes (Trypanroth, blue trypan, afridol…). As the dyes fixe on the micro-organisms, they immobilize or kill them.
1903 is a very important year because H. Wolferstan Thomas (English man) proposes to use an arsenical derivative, Atoxyl, to fight against the African trypanosomiasis. By 1905 he publishes his results in the *British Medical Journal*. The therapeutic of the infectious disease get in then the era of chemotherapy. Discovered in 1863 by Antoine Béchamp (French chemist), Atoxyl is the molecule which leads Ehrlich to the development of the salvarsan or 606.
If one compares the Nobel conference of the two scientists, that of Metchnikoff is in direct connection with phagocytosis, whereas that of Ehrlich treats already arsenical treatment, that is to say: chemotherapy. Chemotherapy arised from work on immunity. The intellectual logic of the magic bullet and the cellular target prevailed on the anti-infectious discoveries of chemotherapy and to decree two Nobel Prize in 1908 was thus based a new discipline which was leaded the revolution the XX^{e} century: the chemotherapy.

Neuronal trafficking of proteins involved in synaptic plasticity: a GFP-based approach

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Background: Number and distribution of ion channels and receptors involved in synaptic plasticity is modulated by several factors including post-translational control of their intracellular traffic. The improvement of molecular biology techniques and the introduction of GFP-based chimeras has boosted our understanding of the molecular determinants of protein sequences that are relevant for selective protein function. We have combined these molecular biology techniques to fluorescence microscopy and videoimaging to analyse the critical steps of assembly, membrane insertion and traffic of small conductance potassium channel type 3 (SK3)and of the p75 neurotrophin receptor.
Methods: Hippocampal cell cultures were transfected with fusion proteins between GFP and different SK3 subunit truncations or P75 GFP-tagged constructs. The distribution of the fluorescent recombinant proteins were analyzed by immunofluorescence confocal microscopy or using a living imaging microscope apparatus.
Results: We analysed full length, truncated versions or mutated constructs of SK3. Qualitative and quantitative image analysis indicated that the full length ion channel distributed in soma, axon and in dendrites, whereas GFPΔ578-736 (deletion of the entire C-terminal domain), GFPΔCaMBD (deletion of the calmodulin-binding site) and GFPΔN (deletion of the N-terminal domain) forms accumulated in the cell body compartment and colocalized with ER marker. The GFPΔ640-736 form (deletion of the distal C-terminal domain) had a distribution similar to control. The N-terminal deleted construct negatively affected transport and assembly of the full length channel.
Movements of intracellular p75GFP were followed by cell imaging at 35°C and found to be sustained by tubulo-vesicular structures acting both anterogradely (0.1-0.5mum/s) and retrogradely (0.1-1.1mum/s), with the retrograde transport characterized by two components.
Conclusions: GFP based approach is important for studying molecular and spatial properties of SK3 channel and p75 receptor in CNS neurons. Our data indicate the presence of molecular determinants within the aminoacid sequence of SK3 protein that are relevant for its intracellular processing; the approach can be extended to the analysis of domains involved in function and pharmacological properties of the channel.

Battling pharmacoresistance in epilepsy patients: pharmacodynamic and pharmacokinetic approaches

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Pharmacoresistance in epilepsy has been defined as resistance to multiple antiepileptic drugs (AEDs) and to AEDs with different mechanisms of action. The causes of drug resistance can be patient-related (i.e. pharmacogenetic), disease-related (type of etiology, disease progression, disruption of the blood-brain barrier, alteration of drug target) and drug-related (i.e. inefficacious mechanisms of action, development of functional tolerance). Immunological mechanisms can also play a role. However, in individual patients it is still unclear what causes their pharmacoresistance.
Research efforts have focused on the “transporter hypothesis”, where altered drug transporters such as P glycoprotein are thought to play a key role, and on the “target hypothesis”, which entails altered sensitivity to AEDs. However, pharmacogenetic studies that have evaluated polymorphisms leading to changed function of P glycoprotein or changed function of drug receptors have thus far mainly yielded negative or contradictory results. A clinical study into the effects of verapamil on carbamazepine resistance is being performed.
The classical approach to pharmacoresistance in epilepsy has been resective surgery and the indications for this kind of therapy have broadened in the last decade. Also, several new AEDs have been introduced in the last 15 years that seem to have improved prognosis somewhat. Novel approaches include studies into the role of the blood-brain barrier disruption, AEDs with novel mechanisms of action and neuromodulation techniques.

Antithrombotics that do not induce bleeding: the “holy grail” found by interfering with von Willebrand factor

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Background: Platelet accumulation at sites of blood vessel wall damage, e.g. as a consequence of the rupture of an atherosclerotic plaque, is the first step in the formation of an arterial thrombus, cause of amongst others myocardial infarction and stroke. Currently used antiplatelet agents are beneficial in the prevention of such events, however without exception suffer from side effects due to an enhanced bleeding risk.
In arterial thrombosis, adhering platelets have to withstand high shear forces induced by the fast flowing blood in stenotic areas, for which they rely on von Willebrand Factor (VWF). VWF is a large multimeric protein present a.o. in plasma, that under normal conditions does not interact with its platelet receptor, glycoprotein Ib. The cryptic GPIb binding site within VWF becomes exposed, when VWF, bound to collagen exposed in the damaged artery, is stretched by the high shear forces. Our starting hypothesis was that in such scenario, interfering with VWF functions would have an antithrombotic effect specifically targeted to the (stenotic) arterial side, leaving haemostasis in the slower vessels unaffected, likely resulting in a lower bleeding risk.
Results: We produced monoclonal antibodies that inhibit either the VWF-collagen or the VWF-platelet interaction, proved their shear rate dependent activity in in vitro flow chambers where blood is pumped at different speeds over a coated collagen surface and determined their epi- and paratope at the atomic level. Finally we tested the antibodies in a high shear arterial thrombosis model in baboons (collab. U Vrystaat, Bloemfontein, SA) where a strong antithrombotic effect was obtained, without prolongation of the bleeding time or increase of blood loss from a standardised incision, and this in stark contrast to currently used antiplatelet agents.
Conclusions: we may have found the “holy grail” of antithrombotic research: a class of compounds with a large therapeutic window between effects on thrombosis and on haemostasis, by interfering with shear-dependent VWF-function. Clearly clinical studies are needed to fully proof that this may open up safe treatment options for especially stroke.

Sculpting the Immunological Response to Dengue Fever by Polytopic Vaccination

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Background: The twin challenges of immunodominance and heterologous immunity have hampered discovery of an effective vaccine against all four dengue viruses. Immunization with one dengue virus is protective against future challenge with the immunizing virus. However, immunity built up after infection by one dengue virus protects only modestly or even negatively against reinfection by the other dengue viruses. In particular, the risk of dengue hemorrhagic fever from one of the dengue viruses during a secondary infection appears to rise significantly if there was a previous primary infection from one of the other dengue viruses. This 'original antigenic sin' implies that an effective vaccine for dengue must induce protective immunity against all four dengue viruses. To date, no such vaccine has been developed.
Methods: We here explore the possibility of using polytopic, or multi-site, vaccination to induce an effective T cell immune response against all four dengue viruses. We investigate whether injection of the epitopes from each of the four viruses in different physical locations sculpts a broader TCR response, by inducing TCR selection for each epitope in different lymph nodes. We determine whether polytopic vaccination reduces immunodominance and increases recognition of the four dengue viruses.
Results: We show that specific lysis against the four dengue strains is super in the multi-site protocol. By physically separating the TCR selection and reducing the pressure on TCR resource competition within each lymph node, the TCR repertoire can be sculpted toward the subdominant epitopes, and so there is a reduction in immunodominance.
Conclusions:
By combining polytopic injection with subdominant epitope priming, a vaccination protocol for sculpting the immune response to dengue is suggested. This new protocol reduces immunodominance more fully that does polytopic injection or subdominant epitope priming alone, both of which reduce immunodominance more than does a traditional four-component dengue vaccine. Subdominant epitope priming followed by secondary polytopic injection of epitopes appears to be a promising vaccination strategy for dengue fever and other multi-strain diseases.

H. Zhou and M. W. Deem, ``Sculpting the Immunological Response to Dengue Fever by Polytopic Vaccination,'' *Vaccine* 24 (2006) 2451-2459

Open Chemical Databases and Ontologies in the Genomic Age

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Modern research in biochemistry and pharmacology depends on availability of chemical data, which until few years ago were almost exclusively concentrated in commercial databases. Not only the scientific community but the humankind as a whole will benefit from open access to chemical data in standard computer-readable format, just as is the case with bioinformatics and genomics. The open access is necessary but not sufficient: for example, patent documents may host a treasure of chemical data, but this treasure is well buried and is not a trivial task to extract even with the cutting-edge text mining techniques.
A number of open-access databases that emerged during the last five years, such as PubChem, ChemSpider and eMolecules, provide free access to millions of structures. However the quality of data (both structure and annotation) depends on community efforts of chemical data curation. The challenges and achievements in the standardisation of chemical language in biological databases will be presented, with emphasis on three aspects of curation:

1) naming: correct, unambiguous and usable nomenclature
2) drawing: unambiguous, computer- and human-readable 2-D diagrams;
3) ontology: linking the entity of interest by defined logical relationships to other entities.

I am going to use the open access chemical databases to illustrate these aspects, with focus on ChEBI, a definitive, freely available dictionary of Chemical Entities of Biological Interest. ChEBI provides standardised descriptions of molecular entities that enable other databases at the EBI and worldwide to annotate their entries in a consistent fashion.
The challenge (1) is illustrated by cases of conflicting nomenclature systems, such as Preferred IUPAC Names (PINs) and International Nonproprietary Names (INNs). Challenge (2) can be met by software implementation of the IUPAC recommendations of graphical representation of chemical structures. The formalization of chemical ontology remains to be addressed. ChEBI ontology, like other Open Biomedical Ontologies (OBO), is manually built with only limited validation. However, the fundamental difference between chemical ontology and biological ontologies is that the former can be formalised using the features derived from connectivity tables. In order to be usable by wider scientific community, the chemical ontology should be scalable so the new compounds could be automatically assigned the ontological relationships.

***N,N*-bis(alkanol)amine aryl esters and *N,N*-bis(cyclohexanol)amine aryl esters: identification of a new class of Pgp-dependent multidrug resistance (MDR) reverters endowed with potencies in the nanomolar range**

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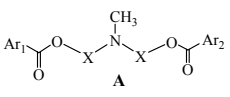
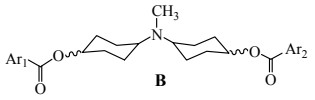
Background: Multidrug resistance (MDR) is an acquired drug resistance of cancer cells and microorganisms to a variety of chemotherapeutic drugs. Classical MDR derives from the over expression of proteins such as P-glicoprotein (Pgp), that act as extrusion pumps using ATP as energy source. Inhibition of the functions of Pgp is considered a suitable approach to circumvent MDR, even if no drug has been yet approved for therapy. All information collected so far on the structure of Pgp point to the existence of a large, polymorphous drug recognition domain, where a variety of molecules can be accommodated in a plurality of binding modes.
Methods: We have obtained a series of molecules where a basic linker tethers, at different distances, two aromatic moieties (A), and a second series of compounds where the flexible moiety of the series A was substituted with a *N,N*-bis-cyclohexylamine scaffold (B), giving origin to a series of geometrical isomers, that represent restricted conformation analogs of the A compounds. The MDR modulating activity was measured by monitoring the pirarubicin uptake on anthracycline-resistant erythroleukemia K562 cells. Selected compounds were tested for their doxorubucin citotoxicity potentiation (RF: reversal fold) on the same cells.



A



B

Results: Flexible derivatives A generally show a good efficacy with potencies ranging from 1.0 to 0.10 μM. Some of the B compounds are even more interesting, the best one presenting an efficacy close to 1, and a potency of 0.012 μM. This compound shows also the best profile in the RF test (36.4).
Conclusions: Applying the frozen analog approach to a series of flexible MDR reverters we have identified a new series of drugs that show very low nanomolar potency and high efficacy: one of them is a promising lead for the development of potent and safe MDR reverters.

Effect of *Valeriana officinalis* in [^{3}H]Glutamate Binding

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Background: Valeriana officinalis root extracts have been used as a sedative and anxiolytic for more than 2,000 years. The most accepted theory establishes that Valerian root extracts stimulate GABA inhibitory neurotransmission. Alternatively, relaxation and sleepiness can be produced if Valerian reduces the activation of glutamate receptors; ionotropic (iGlu) and metabotropic (mGlu) receptors. The objective of our study is to determine the effects of Valerian extracts preparations on the excitatory neurotransmission through [^{3}H]Glutamate binding to mGlu and iGlu receptors. We hypothesized that valerian extracts affect the glutamatergic neurotransmission through the selective interaction with mGlu group II receptors.
Methods: Valerian roots were obtained from Pacific Botanicals, Oregon. Valerian was extracted in ultra pure water (~23ºC) and stirred during 1 hour. Aliquots were centrifuged before being analyzed. Assays were done using synaptic membranes of cerebral cortex from female rats of approximately two months of age. The reaction was initiated by the addition of tissue (100 µg protein) to tubes containing [^{3}H]Glutamic Acid (20nM) in a final volume of 500 µL of 50 mM Tris HCl/ 100 mM KCl buffer, pH 7.4. For the dose response curve, valerian extract concentrations of 4ng/ml – 12mg/ml were used. Non-specific binding was determined in the presence of glutamate 1 mM. All samples were incubated on ice for 40 minutes. The assay was stopped by centrifugation for 30 min at 11,000 rpm, then the supernatant is extracted and the pellet washed with 1 mL of ice-cold buffer. After that, the pellets are resuspended and the radioactivity of the samples was quantified in a liquid scintillation counter with 1 mL of scintillation cocktail.
Results: Aqueous valerian extract ($1x10^{-7}$ – $4x10^{-2}$ mg/ml) increase [^{3}H]Glutamate binding up to a maximum of 60%. At 0.05mg/ml aqueous Valerian extracts specifically interact with KA (*$P < 0.05$*) but not NMDA, AMPA, L-AP4 and quisqualic acid. In contrast, DCG-IV and EGLU markedly decreased, 37% (*$^{***}P< 0.001$*) and 26% *($^{*}P< 0.05$)*, respectively, the [^{3}H]Glutamate binding in presence of valerian extracts (400ng/ml - 10mg/ml) demonstrating that there is a high selectivity for mGluII receptor interactions.
Conclusions: 1) The present study demonstrated that Valeriana officinalis extracts selectively interact with mGluII receptors. 2) This selective interaction of Valerian with mGluII receptors may represent an alternative explanation for the anxiolytic properties of this plant.

Inhibition of experimental Sjögren's syndrome through immunization with Hsp 60kDa and its peptide aa437-460 - predicting treatment efficacy using multi-plex biomarker profiling

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Background: The aim of this study was to investigate a potential immunomodulatory effect of heat-shock protein 60kDa (Hsp60) on spontaneous experimental Sjögren's syndrome (SS).
Methods: 7-week old non-obese diabetic (NOD) mice were immunized with eukaryotic Hsp60 or a Hsp60-derived peptide (amino-acid residue (aa)437-460). At 21 weeks of age, non-diabetic mice were investigated for salivary gland inflammation, exocrine function and extraglandular disease manifestations. In addition, biomarker profiles comprising 87 analytes in serum and 75 in saliva were analyzed.
Results: Immunization with Hsp60 and aa437-460 significantly reduced SS related histopathology compared to NOD controls. In addition, 50% of Hsp60 and 33% of aa437-460 injected mice retained normal exocrine function. Both treatments induced similar changes in biomarker profiles. Notably, circulating IFN-γ-induced protein (IP-10) and eotaxin decreased significantly as a consequence of the treatment. Anti-muscarinic m3 receptor (M3R) IgG1, IL-10 and leptin in contrast discriminated best between the different treatment groups. Successful prevention of hyposalivation was accompanied by quantitative alterations in 36 biomarkers, of which 19 inflammatory mediators declined to levels comparable to Balb/c. Low secreted vascular endothelial growth factor (VEGF)-A predicted most accurately successful prevention of hyposalivation. Low salivary granulocyte chemotactic protein (GCP)-2 was identified as the best predictor of normal secretory function across the strains.
Conclusion: Immunization with Hsp60 and its peptide aa437-460 led to inhibition of SS in NOD mice. Comprehensive analyses revealed specific biomarker signatures capable of predicting treatment group and treatment outcome. Molecules involved in inflammatory chemotaxis, neovascularization and regulatory pathways coined the differences displayed by the biomarker profiles.

Synergy between structural stability and DNA-binding controls the antibody production in EPC/DOTAP/DOPE vesicles and DOTAP/DOPE lipoplexes

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Background: The delivery of nucleic acids using cationic lipids as carriers has been a promising area of research since Felgner in 1987 demonstrated its viability *in vitro*. We present a comparative characterization of physico-chemical properties, *in vitro* cytotoxicity and *in vivo* antibody production of surface-bound DNA on EPC/DOTAP/DOPE and DOTAP/DOPE lipoplexes.
Methods: The DOTAP/DOPE (50:50% molar) and EPC/DOTAP/DOPE liposomes were prepared according to the procedure described by Bangham. EPC/DOTAP/DOPE liposomes were frozen, freeze-dried and rehydrated. The complexation with pVAXhsp65 was carried out at a final molar charge rate (+/-) 10 and final NaCl concentration of 0.9%. Characterizations: average hydrodynamic diameter, zeta potential; plasmid integrity, determination of the molar charge ratio for complete DNA incorporation into the lipid structure, morphology, plasmid accessibility; phase transition; *in vitro* cytotoxicity. After 15 or 30 days of mouse vaccination, IgG1 and IgG2a production were evaluated.
Results: The EPC inclusion stabilized the DOTAP/DOPE structure, producing higher phase temperature and lower zeta potential despite a close mean hydrodynamic diameter. Similar morphologies were identified in both structures, but a higher fraction of loaded DNA was not electrostatically bound in EPC/DOTAP/DOPE. EPC also induced a striking reduction in cytotoxicity, similar to naked DNA-hsp65. The proper immune response induced polarized antibody production of the IgG2a isotype, even for the cytotoxic DOTAP/DOPE. However, antibody production was detected at 15 and 30 days for DOTAP/DOPE and EPC/DOTAP/DOPE, respectively.
Conclusions: The *in vivo* antibody production does not correlate with *in vitro* cytotoxicity, or with structural stability alone. The synergistic effect of structural stability and DNA electrostatic binding on the surface of structures explains the immunological effects, and produced the required condition for DNA delivery.

Synthesis of Some New 1,3,4-Thiadiazol-2-ylmethyl-1,2,4-Triazole Derivatives and Investigation of Their Antimicrobial Activities

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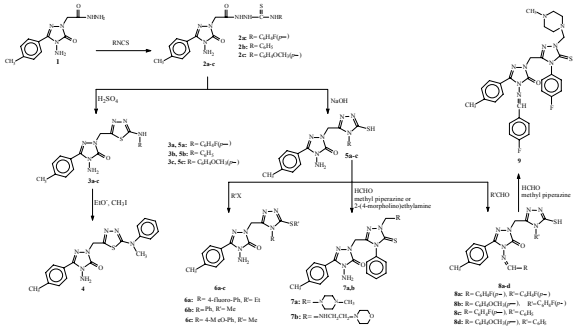
The therapeutic effects of 1,2,4-triazoles have been well studied for diverse patological conditions including inflammation, cancer, pain, tuberculosis and hypertension.
In the past decades, the problem of multi-drug resistant microorganisms has reached on alarming level around the world. For the treatment of microbial infections, the synthesis of new anti-infectious compounds has become an urgent need. For this purpose, several compounds that contain a piperazine or morpholine nucleus possessing antimicrobial activity have been synthesized; some which contains an azole ring also. For instance, while Eperezolid, and AZD2563, which are the members of oxazolidinone class antibiotics, consist of morpholine and oxazolidinone rings bearing with each other via a fluorophenilene linkage, another antibiotic, Linezolid, contains a piperazine ring instead of morpholine. On the other hand, Itraconazole, posaconazole and ketoconazole that are using for the treatment of fungal infections, contains a piperazine and one or more azole ring in their structures.
In recent years, various antitumor drugs have been developed for the treatment of cancer. Among these, some 1,2,4-triazole derivatives incorporating Shiff Base structure were synthesized as antitumor agents in our laboratory. However, cancer is still a major health problem because of the insufficiency of the conventional methods.
Small and linear molecules are suitable for heterocyclic ring syntheses. In this study, some 1,2,4-triazole derivatives were synthesized from the reactions of ester ethoxycarbonylhidrazones (**1**) and screened for their antimicrobial activities. All the newly synthesized compounds displayed IR, ^{1}H NMR, ^{13}C NMR, mass spectral data and elemental analysis consistent with their structures.



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Magical interactions between cisplatin, fluorouracil and radiation benefit oesophageal cancer patients

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Background: "Chemo-radiation" using bolus cis-platinum, infusional fluorouracil with concurrent radiation has been in use since the early 1980s and has resulted in major improvements in outcome for oesophageal cancer patients.

Methods and Results: This presentation reviews the author's 20 years trials experience in this area in the context of experience elsewhere in the world. Issues discussed include:-

- differences in pre-clinical and clinical findings
- dose-response relationships for tumour control and for toxicity
- variations in response relating to gender and age
- the possibility of better combinations of the same agents.

Conclusions: Many of the beneficial interactions between these agents remain incompletely understood and therefore still might be considered "magical". Better combinations of the same agents should be sought before these agents are discarded.

Malaria-induced up and down-regulation of Cytochrome P450: Implications for Pharmacotherapy

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Background: During the last three decades, experimental and clinical studies have shown that infections and inflammatory conditions down-modulate expression and activity of cytochrome P450 enzymes (CYP). A few studies suggested that drug metabolism is depressed in malaria as well. This study was undertaken to extend these observations by investigating the effects of malaria on different CYP isoforms. Additionally, we evaluated whether malaria modulated the effects of alkylating agents including that of an antineoplastic drug.
Methods: Female adult DBA-2 (D2, N=16) and C57BL/6 (BL6, N=19) mice were infected (I) with *Plasmodium berghei* (ANKA) and their parasitemia rates (P) were determined. An equal number of non-infected mice matched for age and sex was the control group (C). Monooxygenase activities (CYP1A: ethoxyresorufin-*O*-deethylase, EROD; CYP2B: benzyloxyresorufin-*O*-debenzylase, BROD and CYP2A5: coumarin 7-hydroxylase, COH) were determined in liver microsomes in mice with P higher than 30% (D2) or 20% (BL6). CYP1A apoprotein levels were evaluated by immunoblotting with an anti-CYP1A antibody. Genotoxic effects (micronuclei in bone marrow cells - BMC) of cyclophosphamide (CPA, 25 mg/kg body wt, CYP2B and 3A- activated), dimethylbenzanthracene (DMBA, 50 mg/kg bw, CYP1A-activated) and ethylmethanesulphonate (EMS, 150 mg/kg bw, direct-acting clastogen) were also investigated in BL6 mice. BMC were harvested 24 h (EMS and CPA) or 48 h (DMBA) after treatment.
Results: Results (mean±SE, ANOVA Dunnett *: p< 0.05, I x C) were in D2: EROD: 60±4.6* x 92±12; BROD: 52±5* x 80.5±7; COH: 170±20* x 93±10 and in BL6: EROD: 53±4* x 118±7.8; BROD: 42.5±5* x 85±11; COH: 11.8±1.6* x 7.9±0.7. Immnublotting showed that levels of CYP1A protein in liver microsomes of infected mice were lower than levels in controls. Data also indicated that malaria attenuated effects of CPA and DMBA and enhanced that of EMS.
Conclusions: 1) Malaria up-modulates CYP2A5 and down-modulates CYP1A and 2B in the liver. 2) Effects of alkylating agents activated by CYP1A, 2B and 3A were depressed while that of a direct-acting agent was enhanced. Taken together these findings suggested that malaria up and down regulates CYP and that it may either increase or decrease effects of drugs depending on the CYP isoforms involved in the activation and or clearance of the compound.

Synthesis, Anti-rhinovirus Activity and Mechanism of Action of New Chromene and Chroman Derivatives

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Background: The human rhinoviruses (HRV) are important pathogens causing most of the upper respiratory tract infections in humans. Although these infections are often mild and self-limiting, the impact on human productivity and on medical costs is enormous. Since more than one hundred serotypes of HRVs make the development of a vaccine impractical, extensive efforts have been focused on the development of effective antiviral agents for the treatment of HRV infections. However, despite the in vitro activity of several compounds, to date only few drugs have shown efficacy in humans and none have been approved for clinical use.
Several flavanoids and flavonoids studied by us exhibited a broad antipicornavirus spectrum. In continuation of the search for more potent and highly selective analogues, we designed, synthesized and tested new (Z)-3-benzylidenechromans, 3-benzyl-2H-chromenes and 3-benzylchromans related to the most active synthetic 3(2H)-isoflavenes and homoisoflavones previously studied by us.
Methods: In preliminary studies, the cytotoxicity of all the compounds was evaluated by measuring the effect on morphology, viability and growth of HeLa (Ohio) cells.The inhibitory activity on HRV 1B and 14 replication was evaluated in a plaque reduction assay, starting from the maximum non-cytotoxic concentration (MNTC). HRV 1B and 14 were selected as representative serotypes for group B and A, respectively. Group B contains twice as many serotypes as group A, and accounts for five times as many colds as serotypes group A.
Results: All the compounds tested showed a potent and selective anti-HRV 1B activity within micro or submicromolar range (IC50s ranging from 0.11 to 6.62 mM). The low cytotoxicities resulted in high therapeutic indexes for all these compounds. In contrast, only a modest inhibition of HRV 14 replication was observed up to MNTC. On the basis of the high activity and therapeutic index (IC50 = 0.12 mM and TI = 625.00, respectively), (Z)-3-(4-chlorobenzylidene) chroman (2b) was chosen to clarify the mechanism of action. The effects of the presence of 2b on different stages of HRV1B growth show that this chroman interferes with an early event of virus life cycle, similarly to previously studied flavanoids. Moreover, the decreased susceptibility of the virus to inactivation by mild acid pH or heat, suggest a stabilizing action of 2b on virion capside conformation.

Platinum and Non-Pt Anticancer Drugs: Insights from High-Level Computations

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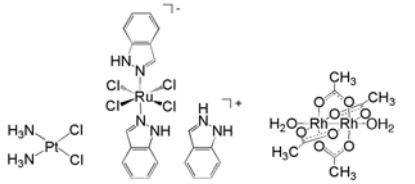
Background: The breakthrough of metals in medicine was the discovery and clinical use of cisplatin as an anticancer drug, which increased the cure rate of testicular cancer from nearly 0 to over 90 percent. The therapeutic spectrum of cisplatin is limited, however, as frequent types of cancer are resistant to the drug or become resistant during therapy. Many patients undergo cycles of partial disease remission, resistance, relapse, and treatment with another drug, until further therapy is considered ineffective. Therefore, the search for new anticancer drugs has continued and extended to compounds of non-platinum metals, most notably ruthenium and rhodium. The importance of high-level computation in these research efforts is rapidly increasing.
Methods: Platinum, ruthenium, and rhodium anticancer agents and their reactions with small models of biomolecules were investigated using computational methods, including quantum chemistry and statistical mechanics for calculating free energies of isolated molecules as well as continuum dielectric methods for calculating energy changes arising from the biological environment.
Results: 1. The computations predict the reactivity of platinum anticancer drugs such as cisplatin towards various functional groups of biomolecules and help identify active drug metabolites. 2. The standard reduction potential (SRP) of ruthenium(III) anticancer compounds such as KP1019, which are likely activated upon reduction to their ruthenium(II) analogs in the hypoxic environment of tumors, can be predicted with a remarkable accuracy of 0.16 V (or 3.7 kcal/mol) using our best computational approach. 3. The complicated molecular mechanism of the binding of the anticancer compound dirhodium tetraacetate to the nucleobases guanine and adenine has been clarified by computation. The mechanism was elusive despite many experimental studies.
Conclusions: High-level computations are complementary to experimental work; they provide promising tools for future guidance of experiments in the development of novel metallo-drugs.



Antifungal therapy in cardiothoracic transplant recipients in a new prescribing era

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Background: Both heart and lung transplantation are established treatments for advanced cardiopulmonary disease. Transplant recipients require life long immunosuppression to prevent allograft rejection placing them at risk of opportunistic infections.
The incidence of invasive aspergillosis in transplant recipients has been reported at 5-20%. Treatment with amphotericin has resulted in patient survival rates of less than 40%. We report a single centre experience of treating fungal infections with the newer antifungal agents.
Methods: We studied 108 patients treated at our hospital from September 2005 to December 2007. Patients requiring inpatient treatment of fungal infections were identified from pharmacy records. Diagnosis required a clinically compatible illness with either the fungus identified in bronchoalveolar lavage specimens, blood or biopsy by microscopy or culture, or radiological evidence of compatible pulmonary lesions after excluding other aetiologies. Outcomes are reported as either successful (*Complete Response:* Resolution in clinical signs and symptoms with regression of radiological lesions. *Partial Response:* Clinical improvement with marked improvement in radiological lesions. *Stable:* Clinical improvement without marked improvement in radiological lesions.) or unsuccessful where patients did not survive to hospital discharge.
Results: 108 patients were treated, 86 lung and 22 heart transplants (annual incidence 29.7% and 3.2% respectively). 61 were male. Age at time of treatment was 46 years (range 19 to 65).Time post transplant was 4.5 yrs (range 2 days to 13 years). 54 patients were taking tacrolimus, 21 ciclosporin and 11 sirolimus. 43 were treated with voriconazole alone and 33 caspofungin alone. 31 were treated with both voriconazole and caspofungin. One was also treated with a third agent (liposomal.amphotericin). Duration of treatment varied from 1.5 weeks to 11.5 weeks. The interaction between voriconazole and calcineurin inhibitors led to peak CNI levels on day 3 of treatment.
87 were successfully treated, 70 lung (81%) and 17 heart (77%). 21 were unsuccessful. In lung transplant recipients, most infections occurred either early(during the first year) (30) or late after 5 years(36). Common symptoms in lung transplant recipients were fever (22) dyspnoea 35, cough 25, purulent sputum 12. 14 patients had a fall in FEV1.
Conclusions: We found a lower mortality from fungal infections treated with voriconazole and caspofungin than that reported with after treatment with amphoterecin. Our data supports a strategy of early and aggressive treatment based on standard criteria.

Aspirin Non-responders in Thai Ischemic Stroke/TIA Patients

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Background: Aspirin resistance has been defined as inability of aspirin to protect individuals from thrombotic complications or to produce an anticipated effect from laboratory tests of platelet function. Most reported information comes from Western patients with coronary artery disease and aspirin resistance is defined by laboratory criteria. The purpose of the study was to look for aspirin non-responders in Thai patients who presented with acute/subacute ischemic stroke and transient ischemic attack (TIA).
Methods: We prospectively included acute ischemic stroke/ TIA patients who were treated at Thammasat hospital during August, 2006- July, 2007 and had already been on aspirin. Information about compliance of medication, reasons for taking aspirin, doses of aspirin, baseline characteristics, stroke subtypes of the patients were collected.
Results: There were 194 acute/subacute ischemic stroke/TIA patients during the study period. Forty-six patients (23.7%), who had already been on aspirin (aspirin non-responder) while having new stroke/TIA, were studied. Eighteen patients were on aspirin 300-325 mg and 28 patients were on 81 mg per day. Most patients have taken aspirin 300-325mg/day as secondary prevention, while half of patients taking aspirin 81 mg/d had diabetes mellitus and took aspirin as primary prevention.
Conclusions: Aspirin non-responders are more common than we previously thought. Future study is required to clarify mechanisms of aspirin non-responders in Thai patients.

Targeting Mutated Janus Kinases In Myeloproliferative Neoplasms Drug Discovery

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Background: Janus kinases (JAK) are protein tyrosine kinases which play a crucial role in controlling many cellular processes; dysregulation of JAK expression and activity leads to different disorders as leukemia and lymphoma, auto-immune diseases, and myeloproliferative neoplasms (MPN). Since 2005, different mutated JAK were characterized. A unique mutation in JAK2, leading to a V617F substitution, is a major molecular event in >95% of the Polycythemia Vera (PV), 50% of the essential thrombocythemia (ET), and 50% of the primary myelofibrosis (PMF) patients. No specific therapy exists against any of these diseases. Especially for PMF, the development of a specific therapy would be useful since the evolution of this disease is usually unfavorable. <30% of PV and ET cases evolve towards PMF. All three can evolve towards acute myeloid leukemia. Thus, the JAK2 V617F offers a molecular target for drug discovery.
It remains a significant challenge to develop selective inhibitors for JAK given their homology and potential structural plasticity. Since the wild type (wt) JAK2 is important for red blood cell formation and for the action of several cytokines and hormones, ideally, an inhibitor should target selectively JAK2 V617F and not wt JAK2.
Methods: Our strategy involved profiling a collection of 1,980 small-molecule compounds from Developmental Therapeutics Program NCI/NIH in a dose–response format against a panel of three JAK dependent cellular assays using growth inhibition screening and overall inhibition of ATP production. Our hits were subsequently analyzed in apoptosis assays, and genetic reporter assay in order to identify better candidates for leads development.
Results: Among 2000 selected small molecules, 7% inhibited proliferation of cells driven by JAK2 V617F, but about 5% also inhibited proliferation of the cells driven by JAK2wt and by JAK1 V658F, a constitutively active JAK1 that harbors the homologous V617F substitution of JAK2. 2% of the compounds showed <5 fold selectivity for the cells expressing JAK2 V617F.
Conclusions: All JAK2 V617F potential inhibitors respect Lipinsky's "Rule of five" and the "New Lead-likeness Rule" and may have potential for further development.

Chloroquine is Therapeutic in Murine Experimental Model of Paracoccidioidomycosis

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Background: Chloroquine, due to its basic properties, has been shown to prevent release of iron from holotransferrin, thereby interfering with normal iron metabolism in a variety of cell types.
Methods: Thus, we have studied the effects of chloroquine on the evolution of experimental paracoccidioidomycosis by evaluating the viable fungal recovery from lung, liver and spleen from infected mice and H_2O_2, NO production, TNF-alpha, IL-6, IL-10 levels by ELISA and transferrin receptor (TfR) expression by flow cytometry from peritoneal macrophages from uninfected and infected mice.
Resuts: Chloroquine caused significant decrease in the viable fungal recovery from all organs tested, during all periods of evaluation. Peritoneal macrophages from chloroquine-treated infected mice showed higher H_2O_2 production and TfR expression, and decreased levels of NO, endogenous and stimulated-TNF-α, IL-6 and IL-10 during the three evaluated periods. Chloroquine-treated infected mice macrophages showed a decrease in NO levels that promoted an increase in TfR expression probably through NO modulatory effect on the iron-regulatory proteins (IRP-1 and IRP-2), which are cytoplasmic proteins responsible for controlling cellular iron storage and uptake by interaction with specific nucleotide sequences, called iron-responsive elements (IREs).
Conclusions: Our findings together supported the idea that therapeutic effects of chloroquine on murine experimental paracoccidioidomycosis is due its capacity to interfere with intracellular iron availability that disabled its uptake by the fungus with consequent fungal death, as well as by its suppressor effects on the macrophage functions. Up to now, no data are available on the possible antifungicidal effect of chloroquine in the clinical situation. Thus, chloroquine could be utilized as a potential drug, which could be administrated in association with conventional paracoccidioidomycosis treatment.
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Cross-Reactivity and Identification of T and B Epitopes in Plant Food Allergens: Peach Pru p 3 as a Model

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Background: Plant non-specific lipid transfer proteins (LTPs) are plant defence proteins that constitute a relevant panallergen family present in both plant foods and pollens. Their high resistance to proteolytic digestion, thus being probably primary sensitizers by ingestion, and association with systemic and severe clinical symptoms, has led to proposed LTPs as a model of true food allergens. Peach Pru p 3, the prototypic member of the family, has been extensively studied at the biochemical, immunological and clinical level.
Methods: Crossreactivity among LTPs was analyzed by specific IgE-quantification and ELISA-inhibition assays. Both sequential and conformational B-epitopes of Pru p 3 were defined by analyzing the IgE-binding capacity of synthetic peptides spanning the full Pru p 3 sequence covalently bound to cellulose membranes, and of a random peptide phage display library (mimotopes), complemented by 3D modelling of the allergen. T-cell epitopes of Pru p 3 were identified by testing the proliferation responses of peripheral blood mononuclear cells and Pru p 3-specific T-cell lines to synthetic peptides covering the entire Pru p 3 amino acid sequence.
Results: Different degrees of cross-reactivity were found between Pru p 3 and other allergens belonging to the LTP family. Comparison of amino acid sequences and 3D structures of these allergenic LTPs suggested the presence of both common IgE epitopes and relevant specific IgE-binding regions different for each allergen. Two principal B-epitopes, comprising residues 31 to 46 and 70 to 80, were located in the Pru p 3-surface. On the other hand, a major T-cell epitope, Pru p $3_{65\text{-}80}$, was identified.
Conclusions: *In vitro* and structural studies have helped to understand cross reactivities among LTP allergens that induce the so call 'LTP syndrome'. The identification of B- and T-cell epitopes of Pru p 3 can accelerate the process to design new vaccines and new immunotherapy strategies for patients suffering such 'LTP syndrome'.

Success story of the first regulatory approval of safety biomarkers, Part II: Consortia, regulatory submissions and translation to human

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Background: A number of promising new renal injury biomarkers have been identified and are now becoming more and more popular not only in the literature but also in pharmaceutical and academic laboratories. Yet these biomarkers are not accepted by health authorities for regulatory decision making such as changing the treatment regimen or selecting patients for treatment.
Methods: To demonstrate the superiority of new urinary biomarkers in comparison to the current standards serum Creatinine and BUN, a consortium was founded (Predictive Safety Testing Consortium, PSTC) and data were exchanged between 16 pharma companies and academia involving 22 pre-clinical studies and 7 urinary renal safety biomarkers in a first round. For a maximum level of compariblity, different consortium standards needed to be created, such as a histopathology lexicon, data processing and statistics. Furthermore, published and contributed clinical data were systematically reviewed to be able to translate the biomarker to human.
Results: The 7 new biomarkers outperformed the current standards for identifying and monitoring tubular and glomerular injury. Together with the FDA/EMEA, a process was defined, how these biomarkers can be submitted for regulatory assessement and approval in a so-called VXDS (Voluntary eXploratory Data Submission).
Conclusions: This story highlights the first successful approval of safety biomarkers by FDA/EMEA and triggered the establishment of standards and of a submission process and qualification process for safety biomarkers. The renal biomarkers submitted were approved by the FDA/EMEA for a regulatory use in a pre-clinical rat GLP study context. In addition, the use of renal biomarkers in clinical trials is to be considered on a case-by-case basis in order to gather further data to qualify their usefulness in monitoring drug-induced renal toxicity in man.

Percutaneous absorption of crotamiton in man following single and multiple dosing.

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Purpose: Crotamiton is a topical drug used in the treatment of scabies and pruritus. We determined its percutaneous absorption following single and multiple dosing in normal skin.
Methods: We used in vivo measurement of percutaneous absorption of [14C] crotamiton in a multidose regimen by measuring urinary excretion and liquid scintillation counting in three groups of four healthy volunteers. The Feldmann urinary excretion method was utilized to ascertain percutaneous absorption. Our results showed that tape stripping does not increase percutaneous absorption of crotamiton; upon repeated application.

Dissociation of Multi-molecular Drug Complexes and Multi-site Binding to 7-TM Receptors: Protection, Delivery and Enhancement of Adrenergic Activation by Ascorbate.

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Using capillary electrophoresis (CE), we developed a new method of measuring molecular dissociation constants. Molecular complexes will dissociate in an electric field. The relative concentrations of ascorbate (Asc), norepinephrine (NE) and an Asc-NE complex were detected using CE in the 0-200 V/cm range. By varying the relative concentrations of ascorbate (Asc) and norepinephrine (NE), a dissociation constant at a constant electric field, the Ke, was determined. Extrapolating multiple log Ke's to 0 electric field generated the Kd for the complex. The addition of ascorbate to NE solutions activating smooth muscle shifted the NE dose-response curve to the left by 0.5 log units, indicating enhancement of NE activity by ascorbate. Since binding of NE by Asc would decrease the free NE concentration, the Asc must dissociate from NE prior to binding. The electric field generated by the cell membrane will exceed 200 V/cm for 8-9 nm from the membrane, spanning nearly the entire space between adjacent cells, thus causing dissociation of Asc-NE complexes in the interstitial fluid. Protein receptors create an electronic shadow protecting the agonist binding site from electric field dissociation. Asc binds to adrenergic receptors in the iM Asc range, binding to a site between the first and second extracellular loops and trapping the adrenergic molecule in its active site, thus effecting the enhancement. This process enhances both alpha and beta 2 adrenergic receptors, increasing contractions of blood vessels and the relaxation of bronchioles, and has been shown to effectively enhance treatment for asthma conditions in horses and sheep. Similar results have been found for Asc enhancement of histamine smooth muscle activation. This finding that both molecules in a molecular complex can both bind to a receptor and maximize that receptor's activity may be a general phenomenon. Membrane electric fields will dissociate any non-covalent complexes as they approach the membrane.

Role of the cell envelope in the antibacterial activities of cyclic polypeptides against *Escherichia coli*

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Background: The role of membrane permeabilisation and disruption in the mechanism of action of some polymyxin analogues against Gram-negative organisms is contentious. The effects of polymyxin B (PMB) and its analogue polymyxin B nonapeptide (PMBN) on *Escherichia coli* envelope integrity should correlate but previous work by other workers suggest different modes of action.
Methods and Results: This work has reassessed the biochemical techniques used before, and shown that in contrast to previous studies, PMBN (a well-characterized antibacterial synergist) readily releases periplasmic proteins and LPS from treated *E.coli* at sub-inhibitory concentrations in normal physiological buffer conditions.
Conclusions: We conclude that PMBN when tested with appropriate methodology, closely correlates with the early effects of PMB on the cell envelope of *E.coli* and this study shows that it is now consistent with the accepted interactions of membrane-active agents on Gram-negative cells.

Superoxide Dismutase Activity in the Kidney of Mice Exposed to Acute Cadmium Intoxication: Protective Effect of Magnesium Pretreatment

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Background: Cadmium (Cd) is not a Fenton metal, but it can indirectly increase production of reactive oxygen species. Literature data indicate that short-term exposure to cadmium can decrease the activities of antioxidant enzymes, one of them being superoxide dismutase (SOD), which contains copper (Cu) and zinc (Zn) in its active site and catalyses the conversion of superoxide anion radical to molecular oxygen and hydrogen peroxide. On the other hand, investigations indicate that excessive intake of bioelements, particularly magnesium (Mg), may antagonize cadmium effects. The objective of the study was to determine the effect of increased oral magnesium pretreatment on superoxide dismutase activity in kidney of mice exposed to acute cadmium intoxication.
Methods: Swiss albino male mice were divided into three groups: I - control group - not treated animals; II - Cd group: animals given single oral dose of 20 mg Cd/kg b.w. as aqueous solution of $CdCl_2$; III – Mg+Cd group: mice given orally 40 Mg/kg b.w. as aqueous solution of $Mg(CH_3COO)_2$ 1 h before Cd treatment. The animals were sacrificed by decapitation at 4, 6, 12, 24 and 48 h and superoxide dismutase activity was determined by method of Misra and Fridovich. Statistical analysis was performed using a one-way analysis of variance (ANOVA), followed by the LSD multiple range test.
Results: The obtained results show that acute Cd intoxication induced significantly decreased SOD activity in kidney after 6 (P<0.001), 12 (P<0.05) and 24 (P<0.001) h. In the kidney of mice pretreated with Mg, SOD activities were not altered if compared with control group.
Conclusion: These results imply a positive role of Mg pretreatment on renal SOD activity in the kidney of mice exposed to acute cadmium intoxication. This could be expalined by the fact that under the same experimental conditions Mg had beneficial effect on Cu and Zn kidney content, as we confirmed in our recent investigations: Cd induced decrease of Cu and Zn (which are necessary for SOD function), while Mg pretreatment increased their levels.

WGA Functionalized Chitosan-Ca-Alginate Microparticles for Targeted 5-FU Delivery in Colon Region

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Background: Experimental efforts on the field of peroral anticancer polymer drug delivery systems will probably enable this therapy to become a reality in a near future. In that course, the development of complex drug carriers *i.e.* bioadhesive hydrogel microparticles (MP`s) for local colon delivery is an approach by combining different principles of targeting and controlled release with muco/bioadhesivity of the system. By providing direct binding to mucosal surface and modifying the residence time and drug release rate, active uptake of therapeutics and better therapeutic efficacy might be achieved.
Methods: Using novel one step spray-drying process, enteric coated 5-fluorouracil (5-FU) nanoparticles were incorporated within chitosan-Ca-alginate network in order to enhance the delivery and residence time at targeted site of GIT, simultaneously avoiding the early drug leaking till reaching the site of action. Prepared particles were further functionalized with WGA in order to increase the therapeutic benefit and to improve site-specific 5-FU delivery. Physico-chemical characterization and cell culture transport and efficacy studies were performed.
Results: Particles with diameter of 8.5μm, high encapsulation efficiency (75.5%), positive surface charge and pH dependent swelling were prepared. FTIR and DSC scans suggested that 5-FU was effectively nano-encapsulated and probably entrapped into MP`s at a molecular level. *In vitro* studies using Caco-2 cells demonstrated the feasibility of MP`s to affects the transport of 5-FU across the cell model, leading to pronounced presence of 5-FU into the cells. By incorporation of 5-FU into MP`s and further functionalization, the uptake of *[methyl-^{3}H] timidine* was reduced for about 30% in comparison with 5-FU solution. These observations could be attributed to improved MP/cell interaction due to the cytoadhesion of the carrier and enhanced tissue accumulation of 5-FU when delivered from MP`s.
Conclusion: Prepared formulations could be suitable candidates for controlled colon-specific delivery of 5-FU, opening a new therapeutic potential for this carriers for local treatment of colon cancer.

Antibiotic Resistance of Food Chain Related Bifidobacteria

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Background: Representatives of the genus *Bifidobacterium* play an important role in the human and animal gastrointestinal microflora. The bifidobacterial microbiota is site and host-specific and of beneficial relevance. Therefore several probiotic bifidobacteria strains were studied and are due to their established health benefits of importance in the food and feed industry.
The use of antibiotics for prophylactic and therapeutic purposes in humans, animals and agriculture has led to an increase in antibiotic resistance in bacteria and the absence of acquired antimicrobial resistance has become an important criterion to evaluate the biosafety of bifidobacteria used as industrial starter or probiotic cultures. As there are no established standard methods available, a large number of isolated bifidobacteria were tested on LSM media (Klare et al., 2005) by using different defined methods, and the MIC data were analysed in relation to the bifidobacterial species.
Methods: A total of about 250 Bifidobacterium strains isolated from the beef and pork production chain were identified by phenotypic and genotypic methods and typed to strain level by molecular methods. The antimicrobial susceptibility against tetracycline, erythromycin, clindamycin, streptomycin, gentamycin, ampilicin and vancomycin was determined by broth microdilution and agar disc diffusion. Resistance genes were detected by PCR-based techniques.
Results: The received data support the need for defining microbiological breakpoints to distinguish between the native population and the resistant suppopulation within each bifidobacterial species. Strains atypically resistant to clindamycin, erythromycin and tetracycline were identified. The PCR-based screening results indicate the presence of *tet*(O), *tet*(W) and *erm*(X) genes. The comparision of the two applied susceptibility testing methods (broth microdilution and agar disk diffusion) showed a clear relationship between the two methods.
Conclusions: For the first time, the antimicrobial susceptibility of a large number of *B. thermophilum* and *B. pseudolongum* isolates from animal origin was studied. The obtained MIC distribution will have some impact on determining the microbiological breakpoint of each tested *Bifidobacterium* species. Overall, isolates originating from a porcine source showed higher resistances than bacteria from a bovine source. This is maybe due to the higher incidence of antibiotic use in swine farming. The assessed susceptibility methods showed interpretable agreement within this study. The disc diffusion zone diameters are highly reproducible and indicate that this method is a useful alternative to broth microdilution for antimicrobial susceptibility screening of bifidobacterial isolates. Hence, some of the analysed strains may act as a potential reservoir for antibiotic resistance genes, which may be explained that these strains have originated from from an environment of higher antibiotic selective pressure.

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Change in Knowledge of Women about Cervix Cancer, Human Papilloma Virus (HPV) and HPV Vaccination due to Introduction of HPV Vaccines

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Objectives. Test knowledge of HPV, cervix cancer awareness and acceptance of HPV vaccination of women now and a year ago.
Methods. Questionnaire were filled out by 305 women visiting 4 gynaecologists of the Regional Hospital Heilig Hart, Tienen, Belgium during two subsequent weeks. Fisher T or Chi² were used as statistical methods to compare the data with the survey of 381 women exactly one year before.
Results. Knowledge about HPV as a cause of cervix cancer and the presence of a vaccine rose from roughly 50% in 2007 to over 80% in 2008 (p<0.0001). Level of education and having daughters, boys or no children were no longer of influence in the level of knowledge or willingness to accept the vaccine. Most parents favour the age group 12-16 years as an ideal time for vaccination. In contrast with the 2007 survey, women below 26 had now acquired almost equivalent knowledge to older women about the virus, cervix cancer and the vaccine, but they were far less likely to accept the vaccine due to its cost price, unless it would be reimbursed (OR 4.2 (1.6-11) p=0.0055).
Conclusion. One year after introduction of the first two HPV vaccines, over 75% of women attending a ambulatory gynaecology clinic know HPV causes cervix cancer and that you can get vaccinated against it. Compared a year earlier, young and lower educated women had dramatically improved their knowledge. However, women below 26 are less prepared to pay the cost price for vaccination if it is not reimbursed.

Do parasitic nematodes regulate cell apoptosis in the host

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Background: Parasitic nematodes turn the immune response towards activation of Th2 and Treg lymphocytes, which create tolerogenic or suppressive immune state in the host. Many infections are chronic and therefore in prolonged time may influence or facilitate the outcome of serious disorders especially when innate immunity or activation of Th1-related response is downregulated. Possibly, regulation of cell apoptosis under ongoing infection is a key phenomenon in duration and intensity of the host immune response. The understanding of mechanisms operated during nematode infection is important to predict the immune reactivity not only to other pathogenic factors but also for treatment procedure.
Aims: 1) To estimate dynamic of cell apoptosis in different phases of nematode infection in mice infected with *Heligmosomoides polygyrus* 2) To evaluate differences in the intensity of apoptosis in slow and fast responder strains of mice 3) To identify stage of nematode which inhibits or induces apoptosis 4) To estimate if pro-apoptotic activity of synthetic glucocorticoid – dexamethasone (DEX) is neutralized by *H. polygyrus* antigens.
Methods: This study included male mice of three strains: middle fast responder mice BALB/c, fast responder mice FVB and slow responder mice C57Bl6 infected with intestinal nematode, *H. polygyrus*. The intensity of cell proliferation, and cytokine production induced by nematode antigens was determined 3, 6, 12, 24 and 30 days post infection Apoptosis of mesenteric lymph node cells (MLNc) *ex vivo* and *in vitro* culture was evaluated by FACS.
Results: During ongoing infection the percentage of apoptotic cells including $CD4^+$ was changed and different in evaluated strains of mice (P<0.0001). In C57Bl/6 apoptosis of $CD4^+$ cells significantly increased in the histotropic phase of infection at 3 day (P<0.0001). In the enteric phase of infection especially on day 12, apoptosis of MLNc was inhibited in each strain of mice. The antigen of infective larvae stage L3 induced apoptosis of MLNc both infected (P<0.05) and uninfected (P<0.05) mice but antigen of the next stage- L4 and adult stage inhibited apoptosis in *in vitro* culture. Adult stages excretory-secretory antigen partially reduced total and $CD4^+$ MLNc apoptosis of uninfected (P<0.0001) and infected (P<0.0001) mice provoked by DEX.
Conclusions: 1) *H. polygyrus* antigens evoked different level of inflammatory reaction in slow and fast responder mice; 2) in C57Bl6 mice a weak inflammation appeared with accordance of accelerated $CD4^+$ cell apoptosis.3) L3 antigens induced apoptosis but L4 and adult worms antigens inhibited apoptosis. 4) The glucocorticoid pro-apoptotic activity was neutralized by *H. polygyrus* metabolic extracts.

The Role of ABC Transporters in the Pharmacokinetics of Miltefosine for Leishmaniasis

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Background: The ATP-binding cassette (ABC) drug efflux transporters breast cancer resistance protein (BCRP) and P-glycoprotein (P-gp) are involved in multidrug resistant cancer. Recently, it was shown that human ABC transporters are possibly also involved in drug resistance of the *Leishmania* parasite by modulating the human macrophage host cell. We investigated whether miltefosine, originally an anticancer drug and now an antileishmanial, is a substrate for BCRP or P-gp and review the literature on this topic.
Methods: The influence of BCRP and P-gp on drug transport, accumulation, and efflux of miltefosine were studied directly in cells overexpressing BCRP1 (MDCKII-Bcrp1), and cells overexpressing MDR1a (LLCPK-MDR1a), grown as a monolayer on a permeable surface. Transport of radioactively labeled was evaluated and compared in transwell-experiments, in triplicate. A review will be given, combining data from literature with our own data.
Results: Miltefosine was not a substrate for BCRP in our cell model. On the other hand, miltefosine was a modest substrate for Pgp (MDR1a). The LLCP-MDR1a cells showed twice as much transport of miltefosine from the basolateral to apical compartment, than vice versa (B-A: 7.5% transport, A-B: 3.4%; means after 4h). A large amount of miltefosine was internalized by the cells. We elaborate on the role of Pgp in the pharmacokinetics and drug resistance concerning miltefosine.
Conclusions: Miltefosine was shown to be a substrate for Pgp, but not for BCRP. Transport was only influenced by overexpression of Pgp (MDR1a) in our transwell experiments. Further studies are warranted to investigate the role of miltefosine in e.g. antimony resistant leishmaniasis.

Adult stem cells are a source of paracrine factors for tissue regeneration.

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Background: Studies in animal models and clinical trails have shown that mesenchymal stem cells (MSCs) participate in the wound healing process in mammalians. Systemically infused MSCs accumulate at the sites of injury and inflammation. Delivery of MSCs to infarcted heart results in improvement of cardiac functions. Only a small fraction of MSCs differentiates into cardiac myocytes suggesting that the major therapeutic effects of MSC delivery are related to release of anti-apoptotic and pro-angiogenic factors
Methods: We investigated interactions of human MSCs with endothelial cells and cardiac myocytes *in vitro*.
Results: We established that MSCs specifically recognize apoptotic endothelial cells. This suggests that endothelial cells undergoing apoptosis may regulate homing of MSCs to the sites of injury. We developed a method to concentrate paracrine factors secreted by MSCs 5-20 times. Concentrated paracrine factors stimulated migration, extracellular matrix invasion, proliferation, and survival of endothelial cells. Co-culture of MSCs with cardiac myocytes improved myocyte survival and triggered mitotic propagation of cardiac myocytes.
Conclusions: We suggest that further investigation of therapeutic properties of paracrine factors produced by MSCs will help to understand the role of MSCs in the wound healing and develop novel therapeutic agents for tissue regeneration.

***L*-tryptophan as a Research Compound and Therapeutic Agent**

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L-tryptophan is a dietary amino acid that has been used as a treatment for mood disorders, either alone or in combination with other pharmacological agents, to augment serotonin function. In the research arena, procedures have been used to reduce L-tryptophan availability and consequently deplete serotonin in the brain, to study the relationship between serotonin and accompanying mood states in participants with various psychiatric disorders. Important findings in this arena over the last decade have produced dramatically increased interest in L-tryptophan research. The clinical benefits of L-tryptophan indicate that it could play a significant role in augmentation strategies in the treatment of psychiatric disorders, and findings from studies that alter L-tryptophan availability further underscore its importance for altering mood states.

Circumventing the blood-brain-barrier. Soviet attempts during World War 2.

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Background. Following the outbreak of the WW2, Soviet scientists were asked to contribute to the war effort. Lina Stern (LS), the first and only female member of the Academy of Sciences (AoS), saw an opportunity to serve her country, while at the same time assessing, if what she had learned in animal experiments with by-passing of the blood-brain-barrier, applied also to humankind. She had noted that direct injection of drugs into the spaces containing cerebro-spinal fluid led to different, sometimes even opposing effects, compared to those seen after injections into blood, muscle or peritoneum. Thus, intracisternal (ic) injections could lead to marked stimulation of autonomic functions.
Methods. Our research is based on the few articles on human patients LS published during WW2 in British and American medical journals, on Soviet publications aimed at informing the international community about scientific achievements in the USSR, as well as on experimental protocols kept in LS' archives at the AoS.
Results. During the war against Finland (1940) and Germany (1941 and thereafter), soldiers suffering from traumatic shock received ic injections of potassium phosphate solutions and a majority benefited from such a treatment. LS was rewarded with a Stalin Prize (1943), although some members of the medical corps of the army remained sceptical of her approach. Significant results were obtained with ic antitoxin injection to tetanos-affected patients, although the data is difficult to interpret, as these patients also received the antitoxin intramuscularly. In 1945-46, LS obtained small amounts of streptomycin, the antibiotic discovered in 1944 in the United States which was the first ever to be active against tuberculosis. She gave it to paediatricians to allow them to treat, with "her method", children affected by meningeal tuberculosis_ which had a very short life expectancy. The "magic bullet" worked, a majority of children survived, however many with hearing deficits, a side effect attributable to the streptomycin.
Coda. Early in 1947, LS was accused of treason and terrorist activity, arrested and detained for nearly 4 years in the abominable Lubjanka prison in Moscow. Whether this was in part due to the unauthorized obtaining (through personal international contacts) and importing of streptomyicn into the USSR remains obscure. More important, however, Staline was turning antisemitic in his last years and the Cold War was looming large. Notice that LS was born into a family of German-speaking, well educated, Latvian Jews. In July 1952, 14 prominent members of the Jewish Antifascist Committee were sentenced : 13 to death, LS to 5 years of internal exile. In 1953, only weeks after Stalin's demise, she could return to Moscow, where she lived until her death in 1968, at the age of 90.

Molecular guidance systems for nuclear-tipped magic bullets: Clinical experiences in thyroid cancer treatment using recombinant TSH

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Background: It is a prerequisite of ^{131}I therapy of differentiated thyroid cancers (DTC) that the sodium iodide symporter be activated in order to maximize iodine uptake. For more than 60 years this was achieved by withholding thyroid hormone replacement for several weeks until thyroid stimulating hormone (TSH) rose into the hypothyroid range. This method of patient preparation is associated with significant side effects in many instances and it prolongs patient time away from work and other normal activities.
Methods: First therapeutic uses were in compassionate care settings with patients who could not be safely rendered hypothyroid (Robbins et al). An international prospective, randomized trial was later performed to compare TSH withdrawal with rhTSH administration. In our own centre, we have also considered the cost implications of the two protocols.
Results: In a retrospective cohort of 115 compassionate care patients it was found that about 40% benefited clinically from rhTSH-stimulated radioiodine therapy. The prospective, randomized trial obtained equivalent clinical outcomes in the two arms at 6-8 months post therapy (Pacini et al, 2006). Further, dosimetry showed that identical administered doses of ^{131}I resulted in a 30% lower whole body radiation dose to those patients who received rhTSH, since euthyroid kidneys maintain function whereas hypothyroidism lowers glomerular filtration rates by 30%. Longer follow up has confirmed that clinical outcomes remain comparable in the prospective study for at least 3 years (Elisei, 2008) and in a separate retrospective cohort to 8.5 years (Rachinsky, 2008). rhTSH use is economically favorable in that time away from employment is reduced.
Conclusions: 1. rhTSH and endogenous TSH are equally effective in preparation of DTC patients for ^{131}I therapy. 2. Further, since circulating radioiodine is excreted more quickly, radiation safety issues are also truncated in time compared to the case of ^{131}I given to hypothyroid patients. 3. In many jurisdictions the cost of rhTSH is an issue for resource-limited health care programs. Cost-effectiveness evaluations show that rhTSH utilization is associated with significantly reduced morbidity, less time away from work and that it is cost-effective in radioiodine therapy protocols.

Interactions of liposomal vesicles with bacterial cells and antimicrobial activity of liposomal antibiotics.

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Liposomes have significant effect as antibiotic carriers on improving drug distribution and decreasing a drug's toxic properties. Liposomal drug formulations were developed to increase the bactericidal efficacy of antibiotics by promoting effective interaction between bacteria and liposomes. Various liposomes containing fluroquinolones and aminoglycosides demonstrated reductions in minimum inhibitory concentrations (MICs) compared with the free drug against Gram-positive and Gram-negative bacteria The antimicrobial activity of PC:Chol:DOTAP cationic liposomes containing meropenem, gentamicin and ciprofloxacin were tested *in vitro* on *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli* strains. Ciprofloxacin loaded liposomes exhibited a 2-4 times higher antimicrobial activity compared with the free drug. The bacterial sensitivity to liposomal meropenem were similar as to free antibiotic. The least effective were the liposomes containing gentamicin. The interactions between cationic liposomal formulations (PC:Chol:DOTAP 3:4:3) and examined bacterial cells were tested by fluorescent microscopy. The study was undertaken because different antimicrobial results had been obtained for liposomal antibiotics. The interactions were examined using PE-Rhodamine-labelled liposomes. Some of the strains exhibited red-light emission (fusion with vesicles or vesicles surrounding the cell) and some showed negative reaction (no red-light emission). The microscopic studies showed interactions of all *Klebsiella pneumoniae* and *E.coli* strains with tested liposomal formulations. Significant variation were noticed for *Pseudomonas aeruginosa* strains. Surprisingly the fusion effect were observed for isolates resistant to liposomal antibiotics. It seems that the efficacy of liposomal drugs strongly dependent on both the outer membrane structure of bacterial cell (interactions that may lead to fusion) and mechanism of bacterial drug resistance. It suggests that if the bacterial resistance mechanism is highly effective even direct drug insertion into the bacterial cell does not significantly change the antimicrobial susceptibility to antibiotics.

Mast cells infiltrate the thalamus as part of the CNS nociceptive response

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Abstract
Mast cells (MCs) accessing brain parenchyma through the blood-brain barrier in healthy animals are limited to pre-cortical sensory relays, the olfactory bulb and thalamus. We have demonstrated that the unilateral repetitive stimulation of the abdominal skin generates controlateral thalamic asymmetry in the distribution of MCs in the rostralmost part of the midline thalamus, the paraventricular pars anterior and reuniens nuclei subregion of animals injected with cyclophosphamide, in strict relation with cystitis genesis. Data are probably related to abnormal visceral/somatic interactions. Thalamic MC asymmetry is restricted to the brain region associated with visceral/vagal inputs, via the nucleus of the solitary tract, and somatic inputs, via the medial contingent of the spinothalamic tract inputs, and takes its origin from abdominal skin where cystitis generates secondary abdominal skin hyperesthesia leading to referred pain in man. We suggest thalamic MCs may play a role in integrative and cognitive sensory processes, including some aspects of visceral pain.

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High Dose Ascorbic Acid in Burn Resuscitation

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Background: Despite improvements in critical care, the resuscitation of patients with burn injuries remains a challenge. Numerous formulas and guidelines have been developed to reduce under- and over-resuscitation, yet they have not been fully successful. Few studies have incorporated the use of adjuncts to address specific mechanisms associated with burn during the early phases of resuscitation. For example, thermal injury is known to be associated with capillary leakage and tissue edema that increases the challenge of fluid resuscitation for treating the developing hypovolemia. It has been postulated that free radical generation associated with thermal injury is an important mediator in the development of this capillary leakage and burn patients are known to present with a reduced antioxidant status.
Methods: Over the past 10-15 years, a series of studies in experimental animals and humans have explored the use of high doses of ascorbic acid in reducing fluid requirements and tissue edema associated with burns. Animal studies have been performed in rats, guinea pigs and sheep at doses as high as 640 mg/kg/24 hr in lactated Ringer's solution. Studies in humans have infused doses of 66 mg/kg. Primary endpoints in all studies have been total fluid infusion and fluid balance. Secondary endpoints have included hemodynamics and antioxidant status.
Results: Studies in experimental animals have reported significant reductions in fluid requirements to achieve equal hemodynamic benefit as long as the vitamin C was infused within 6 hr of the burn injury. Studies in humans reported reduced fluid requirements, less burn wound edema and reduced ventilator days. No overt toxicity was noted in any study.
Conclusions: To date the data suggest that doses up to 66 mg/kg/hr infused for 8-24 hr after burn injury in humans may be effective in reducing fluid needs and tissue edema, and such doses have produced no overt acute toxicity. As an antioxidant vitamin, ascorbic acid has been investigated as a therapeutic agent in several disease states. This presentation will review evidence to suggest that ascorbic acid can be used as a 'magic bullet' as part of early burn resuscitation practices.

Latarcins, Antimicrobial Peptides from Spider Venom: a Variety of the Mechanisms of Action

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Background: The latarcins (Ltc) peptides are present in the venom of *L. tarabaevi* spider and found to possess antibacterial activity, most likely due to their ability to deteriorate plasmatic bacterial membranes. Aim: to determine the mechanism of antibacterial activity of these peptides, the two representatives, the 26-residue peptides, Ltc1 and Ltc2a have been investigated in model membranes of the lipid composition mimicking one of Gram-negative microorganisms (mixture of phosphatidylethanolamine and phosphatidylglycerol, 7:3 molar ratio).

Peptide	Amino acid sequence
Ltc1	SMWSGMWRRKLKKLRNALKKKLKGEK
Ltc2a	GLFGKLIKKFGRKAISYAVKKARGKH

Methods: Using a number of biophysical techniques we have performed: (i) structural study of the both peptides by CD spectroscopy in phospholipid liposomes and by ^{1}H NMR in detergent micelles; (ii) determination of the peptide's effect on the liposomes and planar membranes by a dye leakage fluorescent assay, ^{31}P NMR spectroscopy, and voltage-clamp technique.
Results: The Ltc2a molecule was found to consist of two helical regions (residues 3–9 and 13–21) connected via a poorly ordered fragment. The effect of the peptide on the liposomes suggests the carpet mechanism of the membrane deterioration. In contrast, Ltc1 molecule is featured by an uninterrupted helix within residues 8-23. This peptide affects liposomes weakly, but induces erratic current fluctuations in planar membranes, causing lesions in them.
Conclusions: Ltc family is represented by the peptides exploiting the diverse mechanisms of the membrane perturbation: (i) the carpet mechanism (Ltc2a); (ii) a voltage-dependent pore-forming mechanism (Ltc1). The spider venom, a natural mixture of these peptides, possesses a broad-spectrum antibacterial activity. This implies that mixing the peptides with different mechanisms of activity, is of potential use in the design of effective anti-infectives.

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Pathophysiology of Enteropathogenic E. coli-induced diarrhea: Potential Therapeutic Role of Probiotics

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Background: Enteropathogenic *Escherichia coli* (EPEC) is a gram-negative food-borne pathogen primarily associated with infantile diarrhea. However, the pathophysiology underlying EPEC induced early diarrhea is not fully understood. Diarrhea results from either an increased ion secretion or decreased absorption, or both. Our aims were to test the hypothesis that EPEC infection decreases NaCl absorption (via a coupled operation of Na^+-H^+ exchanger (NHE) and Cl^--HCO_3^- (OH^-) exchangers) in human intestinal epithelial cells (IECs) to cause diarrhea; whereas probiotics act as pro-absorptive agents by increasing NaCl absorption.
Methods: Caco2 cells in culture and mouse models were utilized for assessing the effects of EPEC infection and probiotics treatment. The effect of bacteria at 30 min to 3h on ethylisopropyl amiloride (EIPA) sensitive ^{22}Na uptake and DIDS sensitive ^{36}Cl- uptake was determined. Cell surface biotinylation and confocal microscopy were utilized to assess expression of ion transporters. Real time QPCR was utilized to measure mRNA expression.
Results: EPEC infection markedly inhibited the activities of NHE3 (the predominant sodium absorbing isoform by ~ 50%) and Cl-/OH- exchange activity (50-75%) in Caco-2 cells. The effects of EPEC were dependent upon type 3 secretion system (TTSS) of the bacterium and occurred via EPEC effector molecules: EspF and EspG, respectively. EPEC infection reduced surface expression of apical anion exchanger, SLC26A3 on the plasma membrane (~70%) in Caco-2 cells and in the mouse colon. Treatment of cells with Lactobacillus acidophilus/rhamnosus (LR) stimulated NHE and Cl-/OH- exchange activities and an increase in NHE3 and SLC26A3 mRNA expression in a differential manner.
Conclusion: Our data demonstrate that EPEC infection to human intestinal epithelial cells inhibits NaCl absorption, which may underlie the pathophysiology of EPEC-induced early diarrhea. Our findings also highlight the potential therapeutic roles of probiotics in prevention of diarrhea associated with enteric infections or inflammatory bowel diseases.

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Ganciclovir treatment of infants with cytomegalovirus infection and central nervous system involvement.

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Background: Congenital and acquired human cytomegalovirus (HCMV) infection is very frequent and dangerous especially in the central nervous system involvement. So far vaccination against cytomegalovirus is not possible ,and the search for effective and safe antiviral drug is on. Ganciclovir (GVC) is one of the "oldest "antiviral drugs against HCMV. In The Children's Memorial Health Institute in Warsaw ganciclovir treatment of newborns and infants has been used for several years after the endorsement of the Bioethics Committee and the informed consent of the parents. Aim: Estimation of the efficacy and tolerability of ganciclovir in infants with cytomegalovirus neuroinfection.
Methods: .66 infants at the age from 2 to 12 months with detection DNA HCMV in cerebrospinal fluid by qualitative PCR method were treated with intravenous infusions of ganciclovir. The dose of GCV was established individually after the pharmacokinetic examinations. The longest time of treatment was 12 weeks (3 courses with 1 months interval).The analysis of the blood cell count as well as chemistry were regularly performed. The infants had also neuroimaging and electroencephalographic examinations , were taken into multispecialistic care and followed-up.
Results: Epileptic seizures, hypertonia, chorioretinitis, sensorineural hearing loss, central nervous system malformations, calcifications, hepatosplenomegaly, hepatitis, thrombocytopenia, anemia were clinical symptoms and signs of the cytomegalovirus infection. In all infants, after antiviral treatment cerebrospinal fluid DNA HCMV wasn't found in control. GCV treatment was particularly effective in chorioretinitis, hepatosplenomegaly and thrombocytopenia. After combined antiviral and antiepileptic treatment infants were long term seizures free and even withdrawal of the antiepileptic drugs was possible in 8 cases .Only transient neutropenia was observed in 5 out of 66 infants (7.57%). No other side effects of antiviral treatment were stated during the twelve year follow up (mean 7 years).
Conclusions: 1)Cytomegalovirus neuroinfection treatment with ganciclovir was effective and well tolerated. 2) Side effects of the GCV treatment were mild and transient.

Phenotypization of Cyclosporine A in Stable Renal Transplant Patients

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Background: The immunosuppressive drug cyclosporine A (CsA) shows broad interindividual pharmacokinetic variability due to different intestinal absorption and metabolism. CsA is metabolized extensively in the liver and intestine by the cytochrome P450 3A (CYP3A) to 3 primary metabolites with the AM1 being the predominant. The CYP3A isoenzymes (CYP3A4 and CYP3A5) show function variability between individuals ranging from 7-fold to 10-fold in vivo. Different probe drugs have been proposed for the phenotypization of CYP3A metabolic activity.
The aim of this study was to compare the values of blood CsA levels and its metabolite AM1 during the 12 h interval with the AUC_{0-12} for the phenotypization of CsA.
Methods: 39 stable renal transplant patients on CsA therapy in steady state were included. The mean age was 49 years (49.1 ± 6.3), the mean weight was 76 kg (76.2 ± 7.4), the mean CsA dose was 198 mg (198.4 ± 56.1). CsA dose was adjusted for individual patient so as to reach the therapeutic range measured by trough CsA concentrations. Totally 9 venous blood samples were drawn in each patient – at 0, 0.5, 1, 1.5, 2, 3, 5, 8, 12 h after CsA intake. Blood concentrations of CsA and its metabolite AM1 were measured by means of HPLC with UV detection. Metabolic ratios (MR) of blood concentration CsA/AM1 and the AUC_{0-12} CsA/AM1 were calculated. Spearman´s rank correlation test was used.
Results: There were large intra- and interindividual differences in the MR CsA/AM1 at different blood sample times. In only 10 out of 39 patients (26%) the MR CsA/AM1 indicated the same metabolic group throughout the 0-12 h blood sample time interval. However there were significant correlations between MR CsA/AM1 and MR AUC_{0-12} CsA/AM1 at the particular blood sample time, the strongest correlation was found with the MR CsA/AM1 at 5 h after CsA intake ($r=0.9501$, $P<0.0001$). The MR AUC_{0-12} CsA/AM1 was then divided into 3 metabolic groups with 16 patients having the lowest MR (0.6-1.6) and thus presenting the highest metabolic activity, with 15 patients having MR 1.7-3.1 and 8 patients having the highest MR (3.2-5.3) and presenting the lowest metabolic activity.
Conclusion: Our results show that the MR AUC_{0-12} CsA/AM1 correlated with all the MR CsA/AM1 throughout the 0-12 h blood sample time interval, however the strongest correlation was found with the MR CsA/AM1 at 5 h after CsA intake.

Current knowledge on membrane transporters of vitamin A and its precursors

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Background: Humans must obtain vitamin A (or retinol (ROL)) from the diet either as preformed ROL or as provitamin A carotenoid precursors to maintain some vital functions. Both deficiency and excess of ROL are known to cause pathologies. Thus, a better understanding of the mechanisms of intestinal vitamin A absorption is important to optimize amounts in the diet. Aims: 1) To define mechanisms of absorption of ROL/carotenoids. 2) To focus on potential membrane transporters.
Methods: In presence of oleate/taurocholate, differentiated Caco-2 cell monolayers on inserts are able to produce chylomicrons (CM). ROL and carotenoids were delivered to cells using Tween 40 (0.1%). Glyburide was used at 0.2 or 1.0 mM. Lipoprotein fractions (CMs and VLDL) in the basolateral medium (BM) were isolated by ultracentrifugation. [^{3}H]-Glycerol was used to label newly formed triglycerides and follow CM. Lipids (including retinoids/carotenoids) in cells and media were extracted by solvents, triglycerides isolated by TLC and [^{3}H] counted, and retinoids and carotenoids analysed by HPLC. Inhibitions of SR-B1, NPC1L1, and ABCA1 protein expression were done by siRNAs.
Results: When cells were incubated with ROL for varying times, cellular ROL plateaued within 2h, whereas retinyl ester (RE) formation increased continuously. ROL and RE efflux into BM increased linearly with time; ROL in the non lipoprotein fraction and REs in CM. In contrast to carotenoids, ROL uptake was proportional to ROL concentration (0.5-110μM). ROL efflux into BM occurred via a saturable process at low concentrations (<10μM) and a non-saturable process at higher concentrations. When ROL-loaded cells were maintained on retinoid-free medium, free ROL, but not REs, was secreted into BM. Glyburide significantly reduced ROL efflux, but not ROL uptake. Inhibition of ABCA1 protein expression decreased ROL efflux, but not carotenoid efflux. SR-B1 inhibition did not affect ROL transport, but decreased carotenoid uptake.
Conclusions: 1) ROL enters intestinal cells by diffusion. 2) ROL efflux is partly facilitated, probably by the basolateral transporter ABCA1. 3) Newly-synthesized REs, but not preformed esters, are incorporated into CM and secreted. 4) Carotenoid uptake is mediated by the apical transporter SR-B1 and carotenoid efflux occurs exclusively via their secretion in CM.

Sulfated oligosaccharides as main targets in cruzipain, the major cysteine proteinase of *Trypanosoma cruzi*

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Background: *Trypanosoma cruzi*, the agent of Chagas disease contains a major cysteine proteinase, cruzipain (Cz). This lysosomal enzyme bears an unusual C-terminal domain (C-T) that contains post-translational modifications and most antibodies in natural and experimental infections are directed against it.
Methods: To address the structure of the N-linked oligosaccharides present in the C-T domain, UV-MALDI-TOF mass spectrometry was used in conjunction with peptide N-glycosidase F deglycosylation and high performance anion exchange chromatography. In order to evaluate the immune responses to sulfated moieties on Cz, and the involvement of anionic charged structures in the immune recognition of sulfated glycoproteins, BALB/c mice were immunized with purified Cz and C-T prior and after desulfation treatment.
Results: The MALDI-TOF MS analysis allowed us to identify and characterize a new striking feature in cruzipain: sulfated high-mannose type oligosaccharides. The humoral immune response to sulfates on Cz or C-T was mainly IgG2b. IgG2b reactivity was abolished when desulfated antigens were used as immunogens showing that sulfates are absolutely required for eliciting IgG2b response to Cz. A significant reduction of C-T-specific delayed-type hypersensitivity reaction in C-T-immunized mice was observed when desulfated C-T was challenged, suggesting the involvement of sulfate groups in the generation of memory T-cell responses. Moreover, immunization with C-T elicited ultrastructural abnormalities in heart tissue. Surprisingly, hearts from sulfate-depleted C-T-immunized mice did not show pathological alterations.
In contrast to anti-desulfated Cz mice serum, anti-Cz serum recognized sulfated poligalacturonic acid with relation So_4^{2-}/COO^- = 1(+++); 0.67 (++); 0.4 (+); 0 (-) and Glucose phosphate in a lower degree (+).
Conclusions: We show for the first time 1) the presence of sulfated glycoproteins in Trypanosomatids; 2) that sulfates are able to elicit specific immune responses and appeared to be involved in the generation of heart tissue damage. 3) Our findings suggest that this effect could not be specifically due to sulfates but to anionic charged structures.

Authors' disclosure statement:
Sera from chronically *T. cruzi*-infected subjects with mild disease displayed higher levels of total IgG and IgG2 antibodies specific for sulfated epitopes compared with those in more severe forms of the disease.

Rheumatoid arthritis, *Proteus* and "magic bullets".

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Rheumatoid arthritis (RA) affects over 5 million people in the European community and over 20 million throughout the world.
Stastny's discovery in 1976, that RA patients carry HLA-D4 led to the identification of the "shared epitope" EQR(K)RAA found in HLA-DR1/4 individuals. Over 80% of RA patients belong to the HLA-DR1/4 groups, whilst the frequency of these genes in the general population is about 35%
Immunological and molecular analysis shows that the sequence ESRRAL, which resembles the "shared epitope", is found in *Proteus* haemolysin whilst another sequence in *Proteus* urease crossreacts with type XI collagen found in hyaline cartilage.
Antibodies to *Proteus* bacteria have been found in RA patients from 14 different countries and RA sera have cytopathic properties against sheep red cells coated with EQR(K)RAA peptides.
It would appear that RA is caused by an upper urinary tract infection by *Proteus* bacteria and this would explain why this disease occurs more frequently in women than men.
Therefore antibiotics or Ehrlich "magic bullets" against *Proteus* bacteria should be used as specific therapeutic agents together with non-specific drugs, such as anti-TNF biologicals, DMARDs and NSAIDs in the treatment of RA.
The assessment of specific anti-*Proteus* magic bullets in the treatment of RA is long overdue.

Microemulsions Of Amphotericin B: A Way To Change Its Profile Of Activity And Toxicity

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Background: The intrinsical nephrotoxicity of amphotericin B (AmB), still a drug of choice for antifungal treatment, can be reduced by incorporating it in lipidic carriers. Aims: 1) To develop an AmB microemulsion (ME). 2) To analyze its physicochemical properties. 3)To analyse its *in vitro* pharmacotoxicity.
Methods: The obtained ME system was physicochemically characterized by its visual appearance, refraction index, pH, and particle size. The binding and strength of AmB aggregation to the ME was also evaluated. The *in vitro* pharmacotoxicity of AmB-ME was assessed by using red blood cells (RBC) and *Candida albicans*.
Results: The proposed protocol generated a quite stable ME of AmB (Table 1), which was less toxic and as active as the traditional Fungizon™. The observation of a monomeric band located at 405 nm, along all the concentration of study, revealed that the AmB molecules were strongly and individually bound to the ME droplets.
Table 1. Mean particle size of ME and AmB-ME by photon correlation spectroscopy (Light Scattering)

	Diameter (nm)	Polidispersity index	pH	Refraction index
ME	30.9 ± 2.1	0.218 ± 0.014	6.0 ± 0.0	1.370 ± 0.0
AmB-ME	310.9 ± 20.1	0.361 ± 0.020	7.0 ± 0.0	1.374 ± 0.0

Conclusions: 1) A novel formulation of AmB-ME was prepared by a straightforward and fast procedure. 2) This new formulation presented the same efficacy of the Fungizon™ against *C. albicans* and a lower toxicity against human RBCs. 3) Taken together, these results suggested that ME is an eligible drug carrier for AmB or other water insoluble molecules, and it has potential applications.

Disinfectant effect of Garcinia kola extract on Staph. Aureus

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As the search for newer drugs or other innovative measures for treating or controlling *Staphylococcus aureus* infections intensifies, we investigated the disinfectant effect of *Garcinia kola* on this organism as a hand pathogen. *Garcinia kola* is a plant widely consumed among local people in Nigeria as a stimulant and as a therapy for some ailments such as laryngitis, colics, bronchitis, dysentery and diarrhea. The methanolic and aqueous extracts of *Garcinia kola* seeds were tested in-vitro using the agar dilution method. The minimum inhibition (MIC) was determined as the plate with the lowest concentration on which there was no evident of growth.
The methonalic extract was more effective than the aqueous extract with MIC of 12.5mg/ml. For hand washing studies, the *G kola* activity was compared to hibiscrub (4% chlorhexidine in detergent). Combining *G. kola* extract with soap and water for the hand washing showed significant antimicrobial activity on S. aureus. ($P < 0.05$) and this achieved a 2 log 10 reduction compared to Hibiscrub which achieved a 3 log 10 reduction.
In conclusion, this study shows that crude methanolic extracts of *Garcinia kola* possess antibacterial activity and thus may have a potential role as active biological substances for new drugs against *S. aureus* infections which have developed resistance to most of the antibiotics used for their treatment.

Prediction of species-specific targets for the development of STAMPs based on analyses of bacterial species-level supragenomes

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Background: Current antimicrobial strategies will fail as they rely on broad-spectrum antibiotics that target common cellular functions resulting in the selection and dissemination of genetically encoded resistances that are passed, via horizontal gene transfer (HGT) mechanisms, among pathogens and commensal flora.
Methods: To break this chemical arms race we decided to identify species- and strain-specific targets using 454-based whole genome sequencing and a suite of in-house developed comparative genomic tools to analyze dozens of strains of thehuman pathogens *Haemophilus influenzae, Pseudomonas aeruginosa, Escherichia coli, Shigella flexneri, Staphylococcus aureus,* and *Streptococcus pneumoniae*. In each case we chose strains that were associated with a spectrum of clinical presentations to obtain the most complete species-level supragenome possible.
Results: For all species the core genome (those genes shared among all strains) contained only a minority (20-30%) of the genes present within the species supragenome. Thus, the distributed genome was 2.5 to 4X that of the core genome, and each strain of a species contains a unique set of distributed genes with respect to all other strains. Importantly, each species only carried a small number of genes that were completely unique to that species. On average, for all species examined, the mean number of genic differences for any pair of strains within a species was between 350 & 600 genes, such that 20-33% of each strain's genome is unique as compared to all other strains of the species. Each of the core genes for each species gives a unique phylogeny, proving that HGT is the major mode of evolution and that intraspecific phylogenetic studies are meaningless.
Conclusions: We are using our comparative genomics pipeline together with a suite of annotation and metabolomic programs to identify species- and disease phenotype-specific targets for antimicrobial strategies. We will then develop STAMPS (selectively-targeted antimicrobial peptides) directed towards these diagnostic molecular moieties to specifically target particular pathogens without the risk of broad-spectrum targeting that promotes the rise and spread of antimicrobial resistance cassettes.

Use of retinoids as environmental contamination biomarkers in aquatic ecosystems

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Background: Xenobiotics discharged into the aquatic environment are important to monitor. There are several advantages in using biomarkers instead of traditional chemical analysis. Biomarkers measure integrated responses in time and space and can reveal the nature of the pollutant. Exposure to adverse xenobiotics in wildlife alters retinoid status that decrease reproductive success, altere immune system, and causes dermatologic anomalies and developmental deformities. Aims: 1) To assess damage caused by industrial and human wastes and chemicals intensive uses by agriculture at aquatic and estuarine ecosystems. 2) To use the rates of retinol (R) and retinyl palmitate (RP) as biochemical markers of this environment health.
Methods: The sampling of *Gambusia holbrooki* fish was made in the Fouarat Lake situated in north-east of Morocco and Sebou estuary. At the laboratory (L), a sample was bred and fed under poor retinol diet. Another sample was bred in external basin in natural conditions. The HPLC assays were performed on a Gilson model 307 with using UV/Vis detector at 325 nm. Separation was made using a Nucleosil C-18 column (250 x 4,6 mm). The isocratic elution was realised with methanol:water (90:10, v/v), flow rate 1,6 ml/min for the (R) separation and 100 % ethanol at a flow rate of 1,5 ml/min for the (RP).
Results: (R) determination in the body informs about plasmatic rate whereas the (RP) quantification informs about hepatic retinoid reserve. The comparison of different concentrations in (RP) indicates that fish of the estuary have the highest significant hepatic reserve in comparison with that from other sites. The laboratory's sample possesses the lowest hepatic reserve. (R) concentrations of Fouarat Lake and Sebou Estuary are nearly identical and significantly higher than those of (L) and external bread.

Samples	Fouarat Lake	Sebou Estuary	Laboratory	External breading
Retinol (µg/g)	0.449	0.432	0.275	0.191
Retinyl palmitate (µg/g)	0.228	0.546	0.159	0.288

Conclusions: 1) (RP) and the (R) may be useful as sensitive biomarkers for monitoring chemical contaminants in Gambusia in freshwater and estuarine sites. 2) Use of (RP) and (R) indicate if exposure to chemicals was resperctively chronical or acute. 3) A simple, rapid and inexpensive method was developed for extraction and analysis of (R) and (RP) in Gambusia by reversed phase HPLC.

Plasmodial Plasma Membrane: isolation and its implication in drug transport

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Background: A model to study drug transport across the plasmodial plasma membrane is important to investigate the mechanism involved in drug accumulation and resistance in the malarial parasite. Aims: 1) to obtain parasite plasma membranes purified from *Plasmodium falciparum*, 2) to investigate the role of Pgh1 and 3) to study chloroquine accumulation and ATPase activity in the purified plasmodial plasma membrane.
Methods: To obtain the parasite plasma membranes in the form of vesicles, trophozoites released by saponin treatment from infected-erythrocytes were purified using anti-erythrocyte antibodies fixed to polystyrene beads and biotynylated to facilitate their recovery with a magnetic system prior to disruption by nitrogen cavitation. The effect of chemosensitisers on ATPase activity and chloroquine accumulation capabilities of the plasma membranes were determined. The effect of anti-Pgh1 antibodies on chloroquine accumulation was also investigated. Pgh1 was identified in plasma membranes isolated from various strains of *P. falciparum* and the subcellular localisation of Pgh1 in infected-erythrocytes was examined.
Results: Subcellular localisation of Pgh1 indicated that this protein is present in *P. falciparum* plasma membrane but no link between the overexpression of Pgh1 and chloroquine sensitivity of the *P. falciparum* strains tested could be found. In addition, polyclonal antibodies directed at Pgh1 were unable to inhibit chloroquine accumulation in purified plasma membranes, suggesting that Pgh1 is not involved as a chloroquine transporter in the plasma membrane of *P. falciparum*. Verapamil and other agents known to reverse chloroquine resistance by increasing chloroquine accumulation in parasitised erythrocytes did not affect either chloroquine accumulation or the ATPase activity of isolated membranes indicating that resistance reversal do not occur at the plasma membrane level.
Conclusions: a method for the isolation and purification of the *P. falciparum* plasma membrane was developed. This work provides evidence for a verapamil-insensitive site of chloroquine accumulation in the plasma membrane and demonstrated that Pgh1 is not involved in the mechanism of chloroquine resistance at the plasma membrane level.

Chemopreventive and Renal Protective Effects for Epigallocatechin-Gallate (EGCG) and Resveratrol (RSVL) in Human and Animal Cancer Models

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Background: Catechins (like epigallocatechin-gallate, EGCG) and stilbenes like (Resveratrol, RSVL) are plant-derived polyphenols with numerous health benefits. Aims: we evaluated their chemopreventive effects alone or in combination with cisplatin (CP) in the Ehrlich-ascitis-carcinoma (EAC) solid tumor mouse model, and monitored concomitant changes in serum levels of C-reactive protein (CRP), and lipid-peroxides (malondialdehyde; MDA). Further, in the MDA-MB-231 human breast-cancer cells, the apoptotic potential of RSVL was evaluated by measuring the activation of caspase-3/cleavage of proapoptotic polymerase protein (PARP). Lastly, in rats, we verified the capacity RSVL and EGCG to ameliorate the lethal, CP-induced nephrotoxicity and assessed underlying mechanism/s.
Results: EAC-based induction of solid tumor in mice exhibited markedly elevated CRP (11-fold) and MDA levels (2.7-fold). EGCG and RSVL elicited significant, dose-dependent reductions in tumor size (76-98%) and markedly enhanced the chemopreventive effects of CP. These effects were accompanied by reductions in MDA, and CRP levels that correlated well with the antitumor effects (r= 0.9). Further, in human MDA-MB-231 breast-cancer cells RSVL markedly (5-fold) increased caspase-3 activity to induce PARP cleavage and apoptosis. These responses were specifically blocked by inhibiting caspase-3.
Moreover, in rats, CP (10mg/kg) induced nephrotoxicity (2-5-fold rise in serum creatinine/urea levels) after 4days, and globally-induced animal fatalities after 7days. Kidney-homogenates from CP-treated rats displayed significantly-elevated MDA, and -reduced GSH levels. Rats treated with EGCG, but not RSVL, survived the lethal effects of CP, and showed a significant recovery of renal function; while their homogenates had markedly-reduced MDA, and -increased GSH levels. Significant association was detected between creatinine level and each of MDA (r=0.83) and GSH (r=-0.86); thus indicating causal relationships.
Conclusions: 1) In the EAC-mouse model: EGCG and RSVL elicited prominent chemopreventive effects on their own, and appreciably augmented those of CP as well. The extent of tumor progression was highly reflected by CRP levels. 2) In human MDA-MB-231 breast-cancer cells, RSVL markedly activated caspase-3 to cleave PARP and induce apoptosis. 3) In rats, EGCG, but not RSVL, obliterated the lethal CP-induced nephrotoxicity and renal tissue injury by reducing oxidative stress, and upregulating the endogenous antioxidant machinery, like GSH.

Optimizing New Therapeutic Discoveries from Ethnomedical and Ethnobotanical Data

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Increasingly, ethnobotanical and ethnomedical data are being utilized to identify new therapeutic agents. This is because more and more pharmacopeias are being appreciated for their value as acceptable alternatives to conventional medicine, and that methods are now available to validate their worth. It is recognized that many useful botanical remedies are moderate in potency and toxicity, and thus expectations of these yielding new pharmaceutical agents are remote. However in the context of use, the combination of compounds they contain often serves to amplify the totality of their healing effects. In order to optimize the discovery of suitable candidates, several methods can be applied both in the field and laboratory to achieve this goal. These activities, often require collaboration of a variety of scientists, and can result in the development of new botanicals, phytopharmaceuticals or pharmaceuticals. This presentation will review current techniques used in this process and discuss the value of disease-targeting, ethnomedical focusing, ethnobotanical and chemical dereplication, and use of both functional and mechanistic assays.

Process Research on a Key Synthetic Intermediate of Clopidogrel

EMA T

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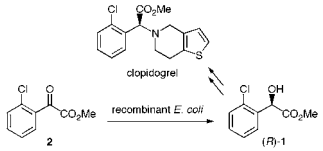
Background: Clopidogrel is a platelet aggregation inhibitor widely administered to atherosclerotic patients with the risk of a heart attack or stroke that are caused by the formation of a clot in the blood. Worldwide sales of Plavix (clopidogrel bisulfate) amounted to $6.4 billion per year, which ranks second. Here we report the environmentally benign and practical chemoenzymatic synthesis of a key intermediate for clopidogrel, (*R*)-**1**, in detail.

Methods: α-Keto ester **2** was prepared according to the literature, and the whole-cell reduction of **2** was done with the recombinant *E. coli*. The results are summarized in Table 1.



Results: The desired alcohol (*R*)-**1** was obtained at 30 ºC in 76% yield with >99% ee (entry 1). Table 1 outlines how we optimized the productivity by changing the substrate concentration and the reaction temperature. When the reaction temperature was decreased by 5 ºC, the conversion and isolated yield increased (entry 2), which prompted us to double the substrate concentration. Even at the substrate concentration of 0.6 M, the conversion reached 94% (entry 3). Therefore, we further increased the substrate concentration up to 1.0 M, which resulted in 90% conversion (entry 4). Finally, we further lowered the reaction temperature (entries 5 and 6) to find the best temperature giving the highest conversion at the same substrate concentration. Thus, the whole-cell reduction of 1.0 M of **2** at 20 ºC gave 99% conversion and 1.78 g of isolated product (*R*)-**1** (entry 5), which corresponds to the productivity of 178 g/L (weight of isolated product per liter of initial reaction volume).

Table 1. Asymmetric reduction of **2** with recombinant *E. coli*

entry	[**2**]/M	[**2**]/g L^{-1}	*T* (ºC)	*conv.* (%)	yield (%)	ee (%)
1	0.3	60	30	92	76	>99
2	0.3	60	25	>99	88	>99
3	0.6	120	25	94	88	>99
4	1.0	198	25	90	85	>99
5	1.0	198	20	99	89	>99
6	1.0	198	15	86	82	>99

Conclusions: In summary, the present biotransformation provides an efficient and green method for the synthesis of methyl (*R*)-*o*-chloromandelate ((*R*)-**1**). The hydride source is glucose, which is the cheap biomass, and the catalyst is *E. coli*, which can be multiplied easily and inexpensively. The reaction is performed in an aqueous solution under air. This is the first example of the direct asymmetric synthesis of (*R*)-**1** with >99% ee. Excellent productivity as high as 178 g/L has been achieved. Because of the pharmaceutical value of the downstream product, clopidogrel, this bioprocess has good potential for an industrial application.

Discovery of Malaria Vaccine Candidates – Application of the HT Cell-Free Protein Production System Born of the Study on Ricin Toxin

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Background: Selection of expression systems plays a crucial role in the post-genomic studies on the structure and function of proteins. Although a variety of cell-based expression systems have been widely used for a long time, they have inherent limitations in terms of their adaptability to high-throughput screening and production, and the quality of the proteins produced. Many of these limitations can be circumvented by the use of cell-free translation systems. Among them, the wheat germ based system is of special interest for its eukaryotic nature; it has the significant advantage of producing eukaryotic multi-domain proteins in a folded state. All of those conventional cell-free systems, however, were plagued by their short lives and as a result, inefficient protein production.

Methods and Results: This shortcoming was overcome by the advent of a new method for the preparation of wheat germ extract, that was based on an idea obtained through my study on molecular mechanism of action of ricin toxin which catalitically inactivates ribosomes. Combining other elementary technologies developed, we established the protocol for the practical use of the wheat cell-free system. The technology consists of (1) in-silico selection of suitable genes from the database, (2) construction of templates for transcription by the split-PCR, and method for (3) transcription and translation reaction. There are two variations of the protocol, one is for genome-wide production and the other is for massive production of protein, have been successfully incorporated into task-specific robots that permit fully automated transcription, translation, and purification overnight.

Conclusions: I introduce how the practical cell-free system is built and it's robotic automation. In addition, I will focus on successful applications to drug discovery such as genome-wide screening of malaria vaccine candidates, the methodology can be extended for selecting effective drugs against mutant enzymes of HIV.

New Strategic treatment for cutaneous leishmaniasis by local injection (Glucantime + triamcinolone 1/20ratio) in 250 patient in Bam-Iran

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Introduction: Leishmaniasis is a protozoal disease whose diverse clinical manifestations are dependent both on the infecting species of leis mania and immune response of the host. 88 countries and more than 350 million people are at risk and the prevalence of this disease is 12 million. ¾ of leishmaniasis presented with cutaneous leishmaniasis.
On 26th of December 2003 a disastrous earthquake occurred at Bam, a city in Iran. After this disaster an epidemic of cutaneous leishmaniasis occurred in this region. Our objective was to study the following:

1. The clinical feature and demography
2. The result of combination therapy by the new way of local injection glucantime and triamcinolone.

Patients and Methods: From December 2004 to March 2006 in a case series study, more than 5000 patients suffering from only cutaneous leishmaniasis (CL) were seen by dermatologists in dermatology clinic of Bam's hospital (Pastor). The clinical diagnosis of CL was confirmed by a direct smear stained by geimsa .Demographic data, including age, gender, residence (Bam or villages), and domestic were recorded in questionnaires.
250 of the patients with less than 3 lesion and had problem for systemic injection of glucantime treated by complete and carefully planed local glucantime injection (Glucantim + triamcinolone 1/20) and pre and post pictures were taken of all the patients.

Results: The mean age of patients was 19y/o. This study of the population included 57% male and 43% female. The locations of lesions in order of frequency were as follows; face hands, upper limbs and feet. The most frequent sites of involvement on the face were the cheeks and then lips, ears and eyelids. The clinical pictures of CL in order of Eczematous and Crusted, Lupoid and recurrent, hyperkeratotic, tumoral, spototrichoid, anthrax like lesions, paronichial lesions, warty and pustular lesions.
More than 72% of patients with eczematous, crusted, lupoid, hyperkeratosis, tumoral and warty form were treated by very careful local injection (G.T 1/20). The plan of injection was as follows:

1. Around the lesion on the outer side of the wound (targeted site) where the skin seems fine up to 0.5cm injected in intradermal as long as we had three signs and symptoms namely: bleaching - hardening - pain and we tried to prevent the leakage of G.T outside the targeted site.
2. Insertion site must be 1 (one) if there was need for more insertions the needle was changed to prevent incubation.
3. We did this twice a week for 6 (six) weeks.

Discussion and Conclusion: Almost of the lesions have three sections namely 1. Necrosis or wound space 2. Granulation tissue (under wound space) 3. Inflammation space around lesion that looks like healthy - this space is important because Leishmania parasites expand in this space, so the most important target by local injection is around this space.
Since CL is an infectoinflammatory disease, low dose of triamcenolone is used to reduce the inflammation and create a more faster and effective working condition for glucontime.
Finally: The correct use of drugs especially local injection of glucontime by the physi side effects of systemic therapy of glucontime and reduce the cost which is beneficial

Natural products from plants and its derivatives as CYP19 (aromatase) inhibitors: flavonoids and dihydroisocoumarin

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Background: Aromatase is a well-established target for the chemoprevention of breast cancer. A part from the development of synthetic aromatase inhibitors (AIs), there is a continuing search for new classes of natural products that inhibit aromatase to discover novel breast cancer chemopreventives.

Methods: CYP19, CYP1A1, CYP2C8, and CYP3A4 inhibition were quantified by measuring the fluorescent intensity of fluorescein, the hydrolysis product of dibenzylfluorescein (DBF), by aromatase. Cell proliferation assay was based on the selective binding of sulforhodamine B with cellular protein (cell lines LU-1, LNCaP, HepG2, and MCF-7). (±)-Abyssinone II and its derivatives were synthetised and the dihydroisocoumarin (3R,4R)-(-)-6-methoxy-1-oxo-3-pentyl-3,4-dihydro-1H-isochromen-4-yl acetate (**1**) was isolated from an EtOH extract of *Xyris pterygoblephara*.

Results: The synthetic (±)-abyssinone II moderately inhibited CYP19 (IC_{50} = 40.95 ± 11.3 μM), whereas (**1**) showed a potent inhibitory activity (IC_{50} = 1.6 ± 0.1 μM). (**1**) did not show inhibitory activity with CYP2C8 and CYP3A4 (IC_{50} > 65 μM) but moderately inhibited CYP1A1 (IC_{50} = 38.0 ± 2.0 μM). Proliferation suppression of LU-1 and HepG2 cells was insignificant, whereas a weak antiproliferative effect against MCF-7 (IC_{50} 66.9 ± 2.3 μM) and LNCaP cell lines was observed (IC_{50} 57.5 ± 2.0 μM). Amongst the (±)-abyssinone II derivatives, 4'-methoxy (IC_{50} = 4.08 ± 2.10 μM), 7-methoxy (IC50 = 4.75 ± 0.61 μM), and 4',7-dimethoxy (IC50 = 3.67 ± 1.61 μM) derivatives were about 10 times more potent than (±)-abyssinone II itself.

Conclusions: The results reported demonstrate that dihydroisocoumarin **1** and the methoxy-(±)-abyssinone II derivatives mediate potent *in vitro* aromatase inhibitory activity. An efficient total synthesis of (±)-abyssinone II and a series of abyssinone II-based flavonoid AIs has been successfully carried out.

Spider silk proteins – a new generation of bioimplantable materials.

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SPIDERMAN CONSORTIUM. 1) Swedish University of Agricultural Sciences, 2) Queen Mary University of London, UK, 3) Sheffield University, UK, 4) Erasmus University, Rotterdam, the Netherlands, 5) Oxford Biomaterials, Newbury, UK, 6) University of Oxford, UK, 7) Q-Med Uppsala, Sweden, 8) University of Twente, The Netherlands, 9) University of Konstanz, Germany

Background; Spider silk consists of fibrous proteins containing highly repetitive sequences of amino acids stored in liquid form that configure into fibres when sheared or spun at secretion. This multipartner project aimed at recombinant production of this elastic tough and tensile polypeptide fibre that can be used for development of implantable surgical devices.
Methods; This study has used a vast range of recombinant technology structural chemical and immunological methodology in combination with a range of in vitro and in vivo approaches.
Results: We have produced strong tensile fibres originating from a partial cDNA sequence from the *Euprosthenops* Spidroin I gene with biomechanical properties that allow the fibres to be used for the development of implantable surgical material.
In parallel progress has been made on the development of alternative material originating from silkworm silks. This class of materials can be used as a scaffold for in vitro synthesis of certain artificially produced biological tissues. Moreover, it has been shown that the different silks form scaffolds that support differentiation and growth of mesenchymal stem cells. .Furthermore biocompatibility parameters of *bona fide* , reconstituted and recombinant silk will be presented. A novel in vitro system for assaying immunogenicity as measured by release of selected interleukins from cultured macrophages has been developed. The characterisation of spider silks ability to release pyrogens and interleukins from target cells has also been deployed. It has been demonstrated that biosynthetic fibers from *Euprosthenops* and refined silkworm silk fibers are can be virtually devoid of LPS contamination and show a promising performance in vivo.
Conclusion: Novel fibrous protein biomaterials have been developed that possess the required biomechanical properties to proceed into the development of implant prototypes.

The Cancer Cell Life-Cycle: Providing Mechanism for Genotoxic Resistance

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Background. Recently, the genetic mutation theory of cancer has been complemented with the concept of "cancer stem cells". This concept recognises that only a small fraction of tumour cells appears to possess the properties of self-renewal and unlimited proliferation necessary to explain our observations of cancer treatment, resistance and re-growth. Two groups of ontogenetic genes appear to be up-regulated in parallel to the degree of malignancy and poor patient prognosis: cancer testes-associated genes and embryonal-germ-line-specific genes, the both may have relevance to the poorly known biology of cancer stem cells. Their importance in resistance to genotoxic stress remains to be fully elucidated. Over eight years ago, we observed the role of transient polyploidy in the genotoxic resistance of p53-deficient lymphoma cells (Illidge et al., 2000; Erenpreisa and al., 2000). Our next task was to see if the above mentioned ontogenetic genes are associated with transient polyploidy.
Methods. Irradiated human lymphoid cell-lines. RT-PCR, Western blotting, and imunofluorescence.
Results. We found previously (Kalejs et al., 2006) and extended observations in the present studies that transient polyploids induced by mitotic catastrophe in irradiated p53-mutant tumour cells express several hall-marks of meiosis and undergo reduction divisions producing mitosis-capable para-diploid cells.
Conclusions. The data support our hypothesis (Erenpreisa and Cragg, 2001, 2007) that the ploidy cycle acts as a bridge from one cancer cell generation (akin to a life-cycle) to another. The slippage from mitotic cycle for the ploidy cycle is started by mitotic catastrophe. Furthermore, we suggest that this life cycle occurs constitutively in tumour cells (affording them immortality through continual rejuvenation of the cancer stem cells) and at an accelerated rate following genotoxic treatment providing them with the ability to survive genotoxic treatments. Therefore, we feel that the next strategy for Ehrlichs' "magic bullets" should target this process.

Biopharmaceutical and Pharmacokinetic Searches of Drugs in Perinatology (1968-2008)

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Background: As it's known the biopharmaceutical factors influence on the pharmacokinetic parameters of medical drugs and therapeutical effectiveness. Since 1968 the aim of scientific-industrial center of biopharmacy of Georgian Ministry of Labor, Health and Social affairs is to work out the Biopharmaceutical and Pharmacokinetic searches of drugs, used in perinatal medicine and obstetric - gynecology to create the effective therapeutical schemes and rational drug forms.
Methods: Our studies worked out by the following directions:
1) To work out the sensitive methods of determination of medical drugs in biological fluids and experimental animals' organs;
2) To implement the permanent method of vaginal and rectal suppositories;
3) To select the release the pain, mastimulate and anti trichomikocidi drugs to create the property material; prepare rational drug forms, investigation their pharmacokinetic and biopharmaceutical data.
For the determination of pharmacokinetic parameters and study the biopharmaceutical factors of drugs we used the following compounds: Aminaloni (^{15}N), Oxybutirat Natrium (^{18}O), Ethmozini (^{34}S) for mass spectral analyze and kavintoni, furazolidoni, ASA and their metabolites, Etazoli and its' modifications, aloe and placenta extracts, "superini", biogenic stimulator – "Amnitini". "Trichomikocidi" and "Alferoli" were studied by chromatofotocolorimetric and spectrofotometric methods modified by us.
Results: Preparations "Supetini" and biostimulator "Placenta extract" is registered by Ministry of Health in Georgia. The rest medical drugs are fulfilled extemporal, confirmed on the base of methodical recommendations and they have use in gynecology, to treat the urological, proctologic, oncological etc diseases.
Conclusions: According to the fulfilled biopharmaceutical, pharmacokinetic and pharmacodinamic searches created about 40 medical drugs mostly in suppositories forms by original permanent methods. Among them "Trichomikocidi" passed clinical approbation and is producing in the Ministry of Health for registration, anti anemia suppositories "Alferol" is in the clinical approbation process, the technical documentations for amnitini suppositories are preparing to get permission for clinical approbation.

Novel Phyto-antiviral Leads from Aglaia Species

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Background: Medicinal plants have consistently served as suitable lead sources for potent antiviral, antimicrobial and other pharmacological agents. The genus aglaia contains more than 100 species, many of which have been shown to possess diverse pharmacological activity. In our current search for novel plant-derived antiviral compounds effective against HIV and other human pathogenic viruses, we focused on 18 triterpenoidal compounds from 5 Aglaia species.
Methods: A total of 18 pure triterpenoidal compounds isolated from 5 Aglaia species (*A. ignea*, *A. duppereana*, *A. cucculata*, *A. euphoroides* and *A. tsangii*) were screened against the human immunodeficiency virus type 1 (HIV-1), human adenovirus type 5 (hAd5) herpes simplex virus type 1 (HSV-1) and the respiratory syncytial virus (RSV) using cell lines permissive for these viruses. Toxicity of the compounds to the cell lines was assessed in parallel using either the MTT method or a standard ATP-based assay. Mechanistic evaluation of the potent compounds was carried out using time-of-addition studies.
Results: While only one compound (dammarenolic acid) displayed very potent and selective activity against HIV-1 and HSV-1, three compounds (dammarenolic acid, aglaiol and niloticin) displayed selective anti-RSV activity. Time of addition studies showed that dammarenolic acid (DA) and aglaiol (AG) targeted both entry and post-entry steps in the viral replication cycle of test virus (against HIV-1 and RSV for DA and RSV for AG).
Conclusions: Dammarenolic acid, aglaiol and niloticin represent novel plant-derived compounds from Aglaia that could be further exploited as suitable leads for the development of potent anti-HIV-1, anti-HSV and anti-anti-RSV agents.

Recombinant Viral Vectors as Suitable Surrogates for Antiviral Screening Studies

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Background: For high throughput antiviral screening studies, safe, rapid, reproducible and cheap screening techniques are needed. Reporter gene-based screening assays are increasingly being recognized as suitable probes for such assays. Here we describe the use of recombinant retroviral and adenoviral vectors expressing either the luciferase (Luc) or green fluorescent protein (GFP) reporter genes as suitable surrogates for the efficient high throughput screening of antiviral compounds from plant or synthetic sources.
Methods: About fifty plant-based and two synthetic compounds were screened for anti-HIV activity using recombinant single-cycle infectious lentiviral vectors expressing either the Luc or GFP gene. To increase tropism the vectors were pseudotyped with the vesicular stomatitis virus Glycoprotein envelop. Infectious lentiviral vector particles generated by transient co-transfection of the vector plasmid with packaging plasmids were incubated with various concentrations of the antiviral compounds and then used to transduce HeLa cells in 96-well plates. Cytotoxicity was assessed in parallel using similar cell lines stably expressing Luc. After two days, the reporter gene read-out of treated cells were analysed and the effect of compounds relative to untreated control was expressed as a percentage. Replication-competent and defective adenoviral type 5-based (Ad5) vectors expressing the Luc or GFP genes were similarly screened with antiviral compounds.
Results: Compounds from Aglaia species, *Ramalina farinacea*, *Jatropha tanjorensis* and *Nymphae lotus* displayed potent anti-HIV activity, with IC_{50} ranging between 2.7 and 18.2 µg/ml. Only selected compounds from Aglaia and *R. farinacea* did show potent anti-Ad5 activity. The anti-HIV effect against the viral vectors was confirmed with 10 times potency against the wild-type HIV-1. The anti-HIV effect and antiretroviral spectrum of activity of nevirapine was authenticated using both wild-type and HIV-1 vector.
Conclusions: 1) The recombinant viral vectors are safe and reproducibly mimick the wild-type viruses. 2) Several plant-based and two standard synthetic compounds were appropriately screened using the vector-based technique.

Magic Bullets and the Nosocomial Nemesis – the KwaZulu-Natal Experience

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Background: The impact of bacterial species, antibiotic drug and hospital type on the empiric therapy of nosocomial infections was evaluated within the public health care system in Kwazulu-Natal.
Methods: A multi-centre surveillance studies instituted in 3 hospitals at 3 progressive levels of health care (district, regional, and tertiary) collected consecutive, non-repetitive isolates commonly implicated in nosocomial infections, viz., *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter spp.* Isolates were subjected to susceptibility testing against antibiotics recommended as empiric therapy for nosocomial infections in treatment guidelines formulated by the National department of Health using the Kirby Bauer disc diffusion method advocated by the CLSI. Percentage susceptibility across (1) bacterial species, (2) antibiotics and (3) hospital levels was statistically analysed.
Results: Susceptibility to antibiotics recommended in the treatment guidelines and hence potentially successful empiric therapy ranged from 5% to 95% with multi-resistance evident in all isolates. Statistically significant differences in overall susceptibility were observed (1) across bacterial species, (2) within 2 of the 3 bacterial species for different antibiotics and (3) across hospital levels for 2 antibiotics with p values <0.001 for (1), ranging from 0.003 to <0.001 for (2) and ranging from 0.001 to <0.001 for (3).
Conclusions: This study showed that the success of empiric therapy would vary depending upon the bacterial species, the antibiotic used and the hospital, thus making a strong case for institution-specific guidelines based on evidence from well-executed surveillance.

Targeting Cellular Mechanisms with Natural Antioxidants from Red Palm Oil in order to enhance Cardioprotection

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Background: Activation of the NO-cGMP pathway is associated with myocardial protection against ischaemia/reperfusion injury. However, high-cholesterol diets alter function of this pathway and these alterations have been implicated in both ischaemic/reperfusion injury and the development of ischaemic heart disease. Little is known about the effects of supplements such as Red Palm Oil (RPO) on the myocardial NO-cGMP-signalling pathway. Aims: 1) to determine whether dietary RPO-supplemention protects against ischaemia/reperfusion injury in rats fed a standard rat chow (control) and cholesterol-enriched diets and 2) if so, to investigate possible mechanisms for this protection.
Methods: Male Long-Evans rats were fed a standard rat chow or a standard rat chow plus cholesterol and/or RPO-supplementation for 6 weeks. Myocardial mechanical function, NO-cGMP signalling pathway intermediates and MAPK phosphorylation were determined before, during and after ischaemia.
Results: Cholesterol supplementation caused a poor aortic output (AO) recovery compared with the control group (35·5 % (SEM 6·2) v. 55·4 % (SEM 2·5)), but when RPO was added, the percentage AO increased significantly when compared with the cholesterol group (63·2 % (SEM 3·1); p<0·05). RPO-supplementation also increased myocardial ischaemic cGMP concentrations. Simulated ischaemia increased intracellular cardiomyocyte nitric oxide levels in the RPO-supplemented group, but not in the control non-supplemented group. Furthermore, we demonstrated for the first time that RPO-supplementation protects the isolated perfused working rat heart during reperfusion from ischaemia/reperfusion-induced injury through a MAPK-dependent pathway.
Conclusions: 1) From our work it is evident that most of the RPO-induced changes occurred during the ischaemic period with the NO-cGMP pathway being a major role player. 2) Our results also suggest that hearts of cholesterol-fed animals were protected through a different mechanism (may possibly include the antioxidant capacity of RPO). 3) The proposed mechanisms include RPO protection in ischaemia via the NO-cGMP pathway and MAPK and PKB/Akt signalling pathways during reperfusion.
It is therefore postulated that RPO might offer an alternative, dietary route to protect the heart against ischaemia-reperfusion induced injury.

The Use of Permeability and Reference Compound Data for Predictions and Understanding of Human Pharmacokinetics.

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Background: Good predictions of human pharmacokinetics (PK) and drug-drug interactions (DDIs) require that permeability (Pe) is considered, the interplay between metabolism and Pe is understood, relationships between Pe vs fraction absorbed (fa) or reabsorbed (freabs) in various important organs have been established, and that reference compound data are available and used.
Methods: Passive *in vitro* Pe and *in vivo* PK-data were collected from the literature. These were used for establishment of Pe vs fa or freabs-relationships in the human intestines, liver, kidneys and brain, and finding suitable reference compounds for drug absorption (intestines, brain and renal), metabolism (liver and gut-wall) and excretion (renal, intestinal and biliary).
Results: Pe vs fa and freabs-relationships were established, and based on these, a Pe-based classification system (PCS) was developed. By combining the PCS and *in vivo* and *in vitro* metabolism and excretion data for reference compounds with known *in vivo* PK-properties, prediction of fa and freabs in various organs, hepatic clearance (CLH), gut-wall extraction ratio (EGW), excretion CL, major elimination routes, DDIs, and drug and metabolite organ/cell retention are enabled. Reference probes include atenolol (for intestinal and hepatic fa, renal freabs, EGW, gut-wall metabolism induction and inhibition potential), metoprolol (intestinal and hepatic fa, CLH, renal freabs, biopharmaceutical Pe-classification), propranolol (CLH and oral bioavailability (F)), verapamil (EGW, CLH, and brain uptake and efflux DDI), midazolam (EGW), digoxin (intestinal, renal and bile excretion CL and DDIs, hepatic uptake, and F), enalaprilat (intestinal and hepatic absorption), rosuvastatin (intestinal excretion CL) and cimetidine (renal excretion CL and PCS Pe-classification). The PCS includes 15 additional substances for Pe-categorization.
Conclusions: Pe vs fa and freabs-relationships (in the PCS) and reference compound data are believed to be very useful for improved predictions and understanding of PK and DDI-potentials in man.

Primary CMF Chemotherapy in Operable Breast Cancer.

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Background: Primary chemotherapy (PC) is becoming an accepted practice for large tumors in order to avoid mastectomies and as a surrogate of outcome.
Methods: a series of 305 patients with tumors > 3cm T2-3/N0-1M0 were treated according to a multimodal approach that consisted on three courses of primary CMF followed by appropriate local treatment and three further courses of CMF or four courses of doxorubicin. Response was assessed by mammography.
Results: the overall response rate was 48% (3% pathological complete response). Conservative surgery was achieved in 79.64% of the patients with a low rate of local recurrences (5%). Toxicity was minimal. With a median follow-up of 104 months, the 8y-DFS was 57.63% and 8y-OS was 67.65%. DFS and OS for cases with clinical response were significantly longer: 70% (p= 0.0048) and 90% (p=0.0042) respectively.
Conclusion: PC with CMF is feasible. A high rate of breast conservative surgery has been achieved. Our results stress the value of PC to increase conservative surgery and as a predictor of outcome.

Synergistic or Antagonistic Interaction between Taxanes and G1/S Arresting Agents in Combination Therapy

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Taxanes (paclitaxel and docetaxel), a novel class of naturally occurring antimicrotubule agents, may represent the most active chemotherapeutic agents developed in the last decade for the treatment of advanced breast cancer and many other types of solid tumors. The promising clinical profiles of taxanes have also promoted considerable interest in combining them with other therapeutic agents. However, accumulating clinical data show that such taxane-based chemotherapy or modality therapy may not always increase the therapeutic efficacy.

Through development of appropriate *in vivo* and *in vitro* model systems, we have evaluation of many clinically used protocols of taxane-based combination therapy. Our results revealed that some of the taxane-containing combination therapy may result in antagonistic interactions so that the actual therapeutic activity produced by two agents is less than their expected synergistic or additive effects. Specifically, our studies show that the combination of paclitaxel or docetaxel with G1-S arresting agents such as 5-fluorouracil, doxorubicin, cisplatin, gemcitabine and gamma-radiation may produce schedule-dependent antagonistic interactions. Further, we have investigated the potential mechanism by which G1-S arresting agents interfere with therapeutic efficacy of taxanes. Data obtained from a variety of assays demonstrated that G1-S arresting agents interfere with the cytotoxic effects of taxanes on both mitotic arrest and apoptotic cell death unless taxanes are administered before G1-S arresting agents. In addition, biochemical examinations revealed that paclitaxel and docetaxel could regulate several apoptosis- and mitotic arrest-related proteins such as phosphorylation of bcl-2, c-raf-1 and activation of NF-kB pathway, but these changes were inhibited when tumor cells were pretreated or simultaneously treated with G1-S arresting agents.

In conclusion, our results indicate that the interaction between taxanes and G1-S arresting agents is highly schedule dependent. Exposure of tumor cells to G1-S arresting agents before taxanes could result in pronounced antagonism. The optimal schedule for this combination might be sequential exposure to taxanes followed by G1-S arresting agents. These findings suggest that careful considerations may be necessary when combining antineoplastic agents that exert their cytotoxic action at different phases of the cell cycle.

Oxystress-induced antitumor therapeutics via targeted-inhibiting heme oxygenase-1 (HSP32) in tumor

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Background: Heme oxygenase-1 (HO-1), which is recently recognized as a heat shock protein (HSP32), play important roles in tumor growth. We have reported that HO-1 inhibitor, i.e., zinc protoporphyrin (ZnPP) exhibited potent anticancer activity, however its poor water solubility hamper its application. To overcome this drawbacks, we prepared water soluble micelles of ZnPP by use of poly(ethylene glycol) (PEG) and styrene-maleic acid copolymer (SMA) (PZP and SZP respecitively) and investigated their physiochemical properties and in vitro, in vivo antitumor effect.
Methods: The molecular size and particle size of the micelles were measured by chromatography and dynamic light scattering. A rat splenic microsomal fraction was prepared for measurement of HO activity, by which the K_i was determined by using Line-weaver-Burk plots. In vitro cytotoxicity assay was carried out by MTT method; in vivo experiments were carried out by use of several tumor models.
Results: PZP and SZP showed high water-solubility (> 200mg/ml). The molecular size of the micelle is about 144kDa, and the particle size is around 60~350nm. PZP and SZP inhibited splenic HO activity in a competitive manner, with the K_i of 0.11μM and 0.15μM, respectively, which is comparable to that of native ZnPP. MTT assay showed dose-dependent cytotoxicity in various cancer cells tested (average IC_{50} of 9μM), whereas normal cells showed realtive tolerance to this treatment. In vivo antitumor experiments clearly demonstrated that PZP and SZP had remarkable antitumor activities, even for the highly malignant tumor-rabbit VX-2 liver carcinoma. In addition, no apparent side effects were observed in this treatment. More important, a synergistic effect of light induced photosensitizing capabilities and HO-1 inhibitory potentials of these micelles was observed both in vitro and in vivo under localized , mild illumination conditions using a xenon light source.
Conclusions: 1) Tumor-targeted inhibition of HO activity, could be achieved by using the micellar HO inhibitor based on EPR effect. Consequently, effective antitumor activity can be accomplished without any apparent toxicity in normal tissues or organs 2) PZP and SZP can also be applied for photodynamic therapy, which will further increase their antitumor activities.

Development of Agonists and Antagonists of Human Thyrotropin Using Site-Directed Mutagenesis and Gene Transfer

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Thyrotropin (TSH) and the gonadotropins (FSH, LH, hCG) are a family of heterodimeric glycoprotein hormones composed of two noncovalently linked subunits, α and β. The hTSH heterodimer was converted to a biologically active single-peptide chain (hTSHβCTPα), by fusing the common α subunit to the carboxyl terminal end of hTSHβ subunits in the presence of a ~30 aminoacid peptide from hCGβ (CTP) as a linker. Ligation of the CTP to the carboxyl-end of hFSH resulted in increasing the biological activity and longivity *in vivo*. In the present study, the hTSHβCTPα, was used to investigate the role of the *N*-linked oligosaccharides of α and β subunits on secretion and function of hTSH. Two deglycosylated variants were prepared: one lacks both oligosaccharide chains on α subunit (hTSHβCTPα_{1+2}), and the other lacks also the oligosaccharide chain on β subunit of the single chain (hTSHβCTPα(deg)). The single-peptide chain variants were expressed in CHO cells and they are secreted into the medium. Absence of the *N*-linked oligosaccharides on α or β *subunits and the O*-linked oligosaccharides on the CTP, does not affect the secretion of the variants. However, the absence of *N*-linked oligosaccharide chain on β decreased the secretion rate of the single-peptide chain. These results indicate that the signal for the secretion exists in the single peptide chain and is independent of the oligosaccharides. hTSH variants lack of the oligosaccharide chains is less potent than hTSHβCTPα on cAMP accumulation and T_3 secretion in human cultured thyroid follicles. Both deglycosylated variants compete with normal hTSH and hTSI in a dose dependent manner. Maximal concentration of hTSHβCTPα_{1+2} (200 μU/ml) decreased significantly the hTSH and hTSI -stimulated levels of cAMP and T_3 secretion. Moreover, the variants significantly inhibited (50%) TSH activity *in vivo,* with respect to thyroid hormone secretion in mice. Thus, this variant, behaves as potential antagonist, who may offer a novel therapeutic strategy in the treatment of Grave's disease, the most common form of hyperthyroidism.

Designing a new agonist of Erythropoietin by Fusing the Carboxyl-Terminal Peptide of Human Chorionic Gonadotropin β Subunit to the Coding Sequence of Human Erythropoietin

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Human erythropoietin (EPO) is a glycoprotein hormone secreted from the kidney and controls red blood cell production. EPO has a wide clinical use in the treatment of anemia associated with renal disease, certain chronic diseases and anemia related to chemotherapy and radiotherapy. One major issue regarding the clinical use of EPO is its relatively short half-life due to its clearance by glomerular filtration. Thus the therapeutic protocol used in the treatment of patient required frequent injections of EPO. Previous studies indicated that fusing the carboxyl-terminal peptide (CTP) of human chorionic gonadotropin β subunit (hCGβ) to human follitropin (FSHβ), hCGα subunit or to thyrotrpin (TSHβ), did not affect assembly, secretion, and receptor binding affinity or *in vitro* bioactivity. However, the *in vivo* potency and circulatory half-life of the proteins containing CTP were substantially increased. Other report indicated that ligation of CTP to FSH is not immunogenic and this construct is already in clinical trials phase 3.

To address the issue of EPO half-life, we constructed a chimeric gene that contains the sequence of the CTP of human chorionic gonadotropin β (hCGβ) subunit bearing four *O*-linked oligosaccharide recognition sites and the coding sequence of human EPO cDNA. Fusing the CTP to the carboxyl-terminal of EPO did not affect secretion, receptor binding affinity or *in vitro* bioactivity. However, both *in vivo* potency and half-life of EPO-CTP were significantly enhanced. A single injection dose (660 IU/kg) of EPO Wild-type (EPO-WT) administered once a week had no significant effect on haematocrit levels. However, EPO-CTP administered as a 660 IU/kg once a week was effective as well as the same total dose of EPO-WT administered as 220 IU/kg 3 times a week. This may emphasize the importance of sustained blood levels rather than total dose of administration for *in vivo* bioactivity. These data established the rational for using this chimera as a long-acting EPO analog. The therapeutic efficacy of EPO-CTP analog needs to be established in higher animals and in human clinical trials.

Neuromuscular blocking drugs and magnesium interactions

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Background: Parenteral magnesium has become an increasingly popular therapeutic agent over the last fifteen years, with established uses in obstetrics, cardiology (especially management of various arrhythmias), anaesthesia and critical care. It has a number of mechanisms of action, including antagonism of the glutamate *N*-methyl *D*-aspartate (NMDA) receptors and calcium antagonism. The latter is involved in inhibition of presynaptic release of acetylcholine (ACh). It is this mechanism that explains its action as a neuromuscular blocking agent and hence its potentiation of neuromuscular blocking (NMB) drugs. Whilst NMBs are a cornerstone of modern balanced anaesthetic practice, one of their principal side effects is persistence into the post operative period – postoperative residual curarisation (PORC). Potentiation of the effects of NMBs from any cause will make PORC more likely.
Methods: The treatment of an elective surgical patient undergoing laparoscopic cholecystectomy who developed rapid atrial fibrillation at the end of the procedure. In order to control the ventricular response rate, iv magnesium was administered.
Results: The patient had recently received NMB drugs and the co-administration of magnesium whilst NMB drugs where still present within the biophase of the neuromuscular junction lead to PORC – with the patient becoming re-paralysed. The patient required sedation and artifical ventilation of the lungs until spontaneous recovery occurred.
Conclusions: This is the first reported recurarisation following the administation of magnesium. Care should be exercised in the use of magnesium if NMBs have recently been administered even if the clinically normal neuromuscular function has returned. The consequences, in addition to severe distress to the patient, include paralysis and loss of airway protection.

Renal Afferent Arteriolar Vasodilator Action of Adenosine Predominantly Involves A2b Receptor Activation

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Background: Adenosine (Ado) plays an important role in regulating renal vascular tone via disparate actions of A1 and A2 receptors. While A2b receptor (A2bR) is expressed in preglomerular vessels, there is less functional evidence regarding the role of A2bR in mediating the vasodilator action of afferent arterioles (AA).
Objective: To determine the role of A2bR in buffering the AA constriction caused by Ado by comparing the effects of A2b and A2a receptor blockade on AA.
Methods: We used the isolated blood-perfused juxtamedullary nephron technique combined with videomicroscopy. A single AA from a rat was visualized and superfused with Ado or Ado agonist, or A2b or A2a receptor blockers (1 rat per experiment).
Results: Ado at 10μmol/L constricted AA (-9.6±2.4%, n=9, p<0.05). In the presence of Ado, SCH, an A2aR blocker, at concentrations of 1, 10, 100, 1000 and 10000 nmol/L elicited only slight decreases in AA diameter from 16.1±0.5 to 15.4±0.5, 15.1±0.4, 14.2±0.3 and 14.6±0.4μm, with maximum effect at a concentrations of 1000 nmol/L (-11.3±3.6%, n=5, p<0.05). Superfusion of Ado treated vessels with MAS, an A2bR blocker, at concentrations of 1, 10,100 and 1000 nmol/L caused greater decreases in AA diameter from 15.7±0.5 to 14.8±0.6, 12.9±0.5 and 12.3±0.4μm (-26.0±4.7%, n=6, p<0.01). Adding SCH 1μmol/L did not significantly augment the Ado mediated afferent constriction elicited by MAS 1μmol/L; however, adding MAS 1μmol/L after SCH 10μmol/L caused further vasoconstriction with AA diameter decreasing by 16.8±2.9 %(from 14.6±0.4 to 12.2±0.3μm, n=5, p<0.01). In response to CV101, an Ado agonist, at concentrations of 0.002, 0.02, 0.2, 2μmol/L, AA diameter increased from 17.2±0.4 to 17.1±0.4, 17.7±0.5, 18.5±0.5 and 20.1±0.6 μm (16.8±2.3%, n=5, P<0.01). In the present of CV101 (2 μmol/L), first superfusion with SCH, an A2a receptor blocker, at concentrations of 1μmol/L, AA diameter decreased slightly from 20.1±0.6 to 18.6±0.6μm (-6.1±0.8%, P<0.05). However, superfusion with MAS after SCH, at concentrations of 1μmol/L, AA diameter decreased markedly to 15.1±1.2μm (-24.2±2.2%, n=5, p<0.01). In addition, in the present of CV101 (2μmol/L), first superfusion with MAS, at concentrations of 1μmol/L, AA diameter decreased signficantly from 20.2±0.6 to 15.4±0.7μm (-24.0±2.6%, n=5, P<0.01). However, superfusion with SCH after MAS, at concentrations of 1μmol/L, AA diameter decreased slightly to 14.6±0.9μm (n=5, p>0.05 via MAS group).
Conclusions: Thus, while both A2b and A2a receptors are functionally expressed in juxtamedullary afferent arterioles, the vasodilator effect of adenosine is predominantly via activation A2b receptors.

Pivotal Evaluation of the Accuracy of a Diagnostic Biomarker: the PRoBE Study Design

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Background: The Early Detection Research Network (EDRN), NCI funded and investigator driven, has the mission to evaluate biomarkers for their clinical utilities in cancer risk prediction, diagnosis, early detection, and prognosis. Abundant cancer biomarkers reported in literature yet few are used in clinics. Therefore, the emphasis of the EDRN is biomarker validation. Although schema for a phased approach to development exists and guidelines are available for study reporting, a coherent and comprehensive set of guideline for a definitive biomarker validation study design have not been delineated.
Methods: We proposed PRoBE study design, Prospective specimen collection and Retrospective Blinded Evaluation, for pivotal definitive evaluation of the accuracy of a classification biomarker. A detailed formulation of all aspects of the design is provided. Four tables itemize aspects that relate to (i) *the Clinical Context;* (ii) *Performance Criteria; (iii) the Biomarker test;* and (iv) *Study power and termination*. Alternative designs and strategies were contrasted to illustrate the merit of PRoBE design.
Results: The ideas are applied to studies of biomarkers the intended use of which is for disease diagnosis, screening, or prognosis. Two EDRN validation studies (breast cancer and prostate cancer) were used as examples to elucidate PRoBE design.
Conclusion: Common biases that pervade the biomarker research literature would be eliminated if these rigorous standards were followed closely. We urge the adoption of the design as standard of practice for pivotal evaluation of the classification accuracy of biomarkers.

Locked and Boweled Over: Infections Down the Line

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Background: Patients who are dependent on total parenteral nutrition (TPN) commonly suffer from recurring central venous catheter related infections (CVCRI) from gram positive organisms. As more CVCRI occur, the available sites for central venous catheter placement diminish. We report a case where linezolid lock therapy was used to prevent CVCRI and prolong the life of the line.
Methods: A linezolid lock solution was prepared by the hospital pharmacy and a new bag was used daily. The patient was trained to administer it himself using aseptic technique. He availed of the 16 h when the line was free and instilled 3 ml of a 2 g linezolid l21 solution (without heparin) into the lumen after 8 h of nocturnal total parenteral nutrition. He was monitored routinely by regular blood culture, line swabbing and testing of platelet levels and inflammatory markers.
Results: In the 21 months prior to linezolid lock prophylaxis, the patient had 18 admissions for CVC-related infections, 28 positive blood cultures and eight CVC changes. He had spent 180 of the preceding 632 days in hospital. In 7 months of linezolid lock prophylaxis, he had one line change and one admission for 7 days due to CVC-related infection and a deep vein thrombosis in the common and external iliac veins. The patient had 44.3 infections per 1000 days (28 line infections in 632 days) prior to lock prophylaxis and 4.5 infections per 1000 days (one infection in the subsequent 221 days) during prophylaxis. The mean time to infection increased from 22.57 to 221 days when linezolid lock prophylaxis was used.
Conclusion: While use of linezolid lock prophylaxis is not suitable for routine prevention of CVCRI, linezolid appears to be an effective method of prolonging the life of a central line in TPN dependent patients when other sites are not available.

Smoking, Streptococci and Sputum

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Background: Smoking is estimated to cause 5 million deaths a year. The contribution of smoking to infection is less well defined than cancer and cardiovascular disease but has been shown in respiratory diseases such as influenza, tuberculosis, Legionnaire's, *S pneumoniae* and other infections such as otitis media, *H. pylori* and human papillomavirus. In 2004, Ireland became the first country to institute a ban on smoking in the work place. This provided an exceptional opportunity to examine the effects of the ban on the epidemiology of respiratory pathogens.
Methods: The numbers of samples positive for *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* isolated from community sputum samples were analysed for a year before and after the smoking ban. Samples from those under 18 were excluded. We obtained monthly smoking prevalence data covering the same period as the clinical data (April 2003 to March 2005) for the Munster area from the Office of Tobacco Control. Data was weighted according to the age and gender distribution of the general population. The before and after totals for each organism were compared as a proportion of the total sputum samples using the chi^2 test. Separate logistic regression models were built for each organism under observation simultaneously adjusting for age group, gender and seasonality.
Results: 1089 and 1095 sputum samples were cultured from the community in the year before and after the smoking ban respectively. There was no significant difference between males and females for any of the organisms studied. The number of isolates of normal oral flora increased from 581 (53%) samples to 623 (57%). There was a non significant reduction in levels of all three organisms during the smoking ban. Interestingly, both *H influenzae* and *S pneumoniae* were reduced by the same amount (1.7%). The change in *S pneumoniae* isolation rates approached significance (p = 0.065). On adjusting for the effects of age and gender in a logistic regression model the odds ratio (OR=0.746) for *S pneumoniae* before versus after the ban became statistically significant (p =0.048).
Conclusions: This study showed a significant difference between *S. pneumoniae* subgroup samples before and after the smoking ban. Smoking is a risk factor for *S. pneumoniae* colonisation and infection. This is the first time that a population reduction in smoking has been shown to reduce community pneumococcal levels.

Structure-Activity Relationships of Some 1,4-Dihydropyridine (DHP) Derivatives Evaluated by Interactions with the Physical Properties of Synthetic Lipid Bilayers and Rat Liver Mitochondrial Bioenergetics

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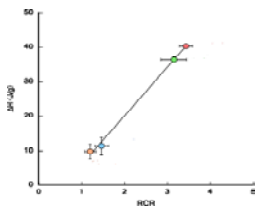
Background: Studies of chemical–biological interactions, particularly, the influence of minor changes of substituent structure, play a major role in the understanding of drug's structure-activity, drug's development and therapeutic success. Aim: to correlate the length of the alkoxyl chain in positions 3 and 5 of the DHP ring of four DHP derivatives (OSI-1210, OSI-1211, OSI-1212, and OSI-3802) with their actions on the physical properties of synthetic lipid bilayers and mitochondrial bioenergetics.
Methods: Biophysical studies were performed by differential scanning calorimetry (DSC) using multilamellar vesicles of dimyristoyl-phosphatidylcholine (DMPC). Rat liver mitochondrial bioenergetics was evaluated by measuring respiratory activities with oxygen and tetraphenylphosphonium (TPP^+) electrodes. All the experiments were performed using four different DMPC and mitochondrial preparations. One-way ANOVA test, followed by the posthoc Tukey test, was used for statistical analysis.
Results: At low concentrations (≤ 30 μM), OSI-3802, like its analogue OSI-1212, reduced the phase transition temperature (Tm), the cooperative unit size and the enthalpy associated with the phase transition of DMPC bilayers. A good correlation was established between the effects of 200 μM OSI-1210, OSI-1211, OSI-1212,and OSI-3802 on the respiratory control (RCR) of rat liver mitochondria and on the enthalpy change (ΔH) for the endothermic profile of DMPC vesicles at 0.2 drug/DMPC molar ratio.



Conclusion: 1) The changes induced by these 1,4-dihydropyridine derivatives on both mitochondrial function and lipid bilayers biophysics are strongly related to the length of the alkoxyl chain in positions 3 and 5 of the DHP ring. 2) This experimental strategy is a good *in vitro* tool to evaluate structure-activity of related compounds, contributing to their synthesis amelioration.

Seropositivity for Hepatitis B Virus, Vaccination Coverage, and Vaccine Response in Dentists from *Campo Grande, Mato Grosso do Sul, Brasil.*

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Background: This study investigated the seropositivity for hepatitis B virus (HBV), the vaccination index, and the vaccine response index in dentists from Campo Grande, MS.
Methods: Blood samples from 474 dentists (63.7% women and 36.3% men), with a mean age of 38.5 +/- 10.5 years were analyzed by enzyme-linked immunosorbent assay to detect the serological markers: HBsAg, anti-HBs, and anti-HBc. The HBsAg positive samples were tested for anti-HBc IgM, HBeAg, and anti-HBe. Viral DNA was detected by polymerase chain reaction in HBV seropositive samples.
Results: A total of 51 (10.8%) dentists showed seropositivity for HBV. Three (0.6%) were HBsAg/anti-HBc/anti-HBe positive, 43 (9.1%) were anti-HBc/anti-HBs positive, and 5 (1.1%) had only anti-HBc. Viral DNA was detected in 9 (17.6%) out of 51 HBV seropositive samples. A vaccination index of 96.6% (458/474) was observed, although 73.1% (335/458) completed the three-dose schedule. Excluding 46 HBV seropositive individuals from 458 that reported vaccination, 412 were analyzed for vaccine response index. It was observed that 74.5% (307/412) were anti-HBs positive; this percentage increased to 79.1% when three doses were administered.
Conclusions: The results showed a high vaccination index and a good rate of vaccine response; however, the failure in completing the three-dose schedule and the occurrence of HBV infection reinforce the need for more effective prevention strategies.

Blocking the Peptide Tunnel- Is the Magic Still There?

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Background: The bacterial ribosome peptide tunnel is the target of many antibiotics, including diverse classes of natural products such as macrolides, chloramphenicol, clindamycin and streptgramins, and the synthetically-derived agent, linezolid. These antibiotics bind to different yet closely located sites creating a "traffic jam" and thereby preventing new peptide synthesis. Bacteria, in the mean time, have gained resistance by methylation of these sites to prevent those antibiotics from binding. Can we improve upon these antibiotics to make new ones?
Methods: Structure-activity relationships within each class of antibiotic, as well as across antibiotic classes that bind to the peptide tunnel have been useful in delineating and mapping the critical regions on the ribosomes that must be blocked to produce new antibiotics with properties that are not seen with any single class of known antibiotic. Novel synthetic chemistry techniques coupled with knowledge obtained from recent structural data on the bacterial ribosome and co-crystal structures were used to design new molecules.
Results: A-60667 and a series of 11-12 carbamate erythromycins were first reported in 1989 to gain activity against bacteria with inducible and constitutive macrolide resistance. This concept was extended by the development of a novel ketolide class, which includes telithromycin, where additional activity was gained by removal of the 3-cladinose sugar and oxidation of the resulting 3-OH group. CEM-101, a ketolide that has a metabolically stable side chain, is more active than telithromycin and is active against macrolide-resistant bacteria. Novel chemistry at the 5-position that allows replacement of the desosamine sugar is leading to molecules that take even further advantage of lessons learned from different classes of peptide tunnel binding antibiotics.
Conclusions: CEM-101 is a new antibiotic that is in clinical development to address infections caused by macrolide-resistant bacteria. Further exploration of the peptide tunnel could provide macrolides with the next big leap in activity and expanded spectrum.

Authors' disclosure statement The authors are employees or advisors of Cempra Pharmaceuticals, Inc.

HIV/AIDS and Malarial/Toxoplasmosis Co-Infections: a Magic Bullet Approach to Drug Development

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Background: AIDS, caused by the virus HIV-1, is not only deadly, but becomes deadlier when the patient gets co-infected with another pathogen such as the malarial parasite *Plasmodium falciparum*, or *Toxoplasma gondii*, the causative agent of toxoplasmosis. The industry approaches to anti-retroviral drug development consist of targeting specific steps in viral entry or replication/maturation. Unfortunately, HIV-1 can quickly mutate to change such target sites, thereby becoming drug resistant. A cocktail of such individual drugs has been more effective, but does not address the problems of co-infections with unrelated pathogens. An ideal drug will be a magic bullet, that will not only simultaneously attack targets in HIV-1, *P. falciparum* and *T. gondii*, but also host factors that act as receptors of viral/parasite entry and growth.
Methods: Two bacterial proteins, azurin and Laz, members of the family called cupredoxins, showed both antiviral and antiparasitic activities. Assays of HIV-1 growth suppression, inhibition of *P. falciparum* parasitemia, *Toxoplasma* adhesion/invasion as well as protein-protein interaction studies have shown the ability of azurin and Laz to inhibit the growth of HIV-1 and malaria/toxoplasmosis-causing parasites, thus acting as a magic bullet.
Results: We demonstrate that azurin and Laz can avidly bind key envelope/surface proteins of HIV-1, *P. falciparum* or *T. gondii,* thereby interfering in their invasion of host cells and growth. Additionally, azurin/Laz strongly binds the host receptor CD4, or the dendritic cell surface protein DC-SIGN that contribute to HIV-1 transport and entry to T cells. Azurin and Laz demonstrate structural features similar to immunoglobulins, thereby implying the possibility of a common evolutionary origin of cupredoxins and immunoglobulin folds. The elucidation of broad target specificity of azurin or Laz towards viruses and parasites appears to suggest that this cupredoxin is used by the producing bacterium as a weapon targeted to other intruders in human body.
Conclusion: Our data show that azurin/Laz acts as a promising magic bullet drug candidate as conceived by Paul Ehrlich, by interfering in several essential steps in HIV-1 entry/growth and also in preventing co-infections by other human parasites.

It's not only genes - The many dimensions of personalized medicine

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Background: The concept of Personalized Medicine is strongly associated with pharmacogenomics. In fact, the term 'personalized medicine' is not occurring in the headings of articles before the advent of pharmacogenomics. Most articles published so far on personalized medicine also contain the term 'pharmacogenomics or -genetics' in their text. However, there is more to it than gene polymorphism.
Methods: Here, it is looked for factors that shape the idiosyncratic way of how health and disease develops in individual human beings. What are the main influences that make us distinct in developing disease and responding to treatment? How should healthcare respond to these individual characteristics in order to make medicine really personal?
Results: On top of the genetic background, three main factors form our health-related individuality: the individual environment, the individual immune system, and our personal lifestyle. It is mainly the microbiological environment that challenges our health, but also nutrition and toxic products of the environment play their roles. The immune system learns how to cope with the microbial challenges and our personal lifestyle strongly influences the spectrum of environmental threats we encounter. These factors - genes, environment, immune system, and lifestyle - are not independent but highly intermingled.
For healthcare to successfully respond to these challenges it has first and foremost to be aware of them, and then to find personal solutions. Both require an individual information management that brings together patient-specific and knowledge-based information in order to make the right personal decisions. Finding the most effective personal treatment with minimal side-effects is an important target of personalized medicine, but genetic counseling and personal patient education is equally important on the long term in order to prevent further disease. Also privacy, protection of minorities, and prevention of discrimination have to be considered.
Conclusions: Personalized medicine is multi-dimensional. Since these dimensions are intermingled, only integrated healthcare based on effective information management will be successful. Prevention, diagnosis, treatment, and care will have to be integrated in order to leverage personalized medicine. The goal is magnificent: personal health planning, early diagnosis, the right drug for the right patient, and predictable side effects.

The Pharmacokinetics (PK) and Pharmacodynamics (PD) of Platinum (Pt) Analogs in Birds

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Background: Chemotherapy as a treatment modality is increasingly being used in avian oncology. However, most chemotherapeutic agents, like Pt analogs, can only be used empirically as PK data in birds is lacking. Aims: 1) To study the PK/PD of cisplatin and carboplatin in cockatoos (*Cacatua galerita*) so that clinical trials can begin.
Methods: This study was done on 2 groups of 6 healthy cockatoos. One group received a single 1 h intravenous (IV) infusion of cisplatin (1 mg/kg), while the other group received a single 3 min IV or intraosseus (IO) infusion of carboplatin (5 mg/kg). For cisplatin, birds were hydrated before and 2 h after the infusion. Serial blood samples were collected for 96 h and urine samples were collected during the 2 h hydration period. Tissue samples from 10 organs were obtained at necropsy (96h). Total and filterable Pt in plasma, and urine and tissue Pt were assayed by inductively coupled plasma–mass spectrometry. A noncompartmental pharmacokinetic analysis was performed on the data.
Results: For cisplatin, the respective mean systemic clearances (Cl) for total and filterable Pt were 373 and 699 mL/h/kg, the steady state volumes of distribution (Vss) were 4.19 and 0.356 L/kg, and the mean residence times were 111 and 0.512 h. Total plasma Pt displayed a bi-exponential decay profile with average half-lives ($T_{1/2}$) of 0.398 and 79.0 h, while filterable Pt had a monoexponential decay with mean $T_{1/2}$ of 0.413 h. The renal clearance was 0.167 L/h/kg. For carboplatin, the maximum plasma filterable Pt concentration of 27.3 mg/L occurred at the end of the infusion, thenceforth declining exponentially over the next 6 h. The terminal $T_{1/2}$ was 1.0 h, Cl was 330 mL/h/kg and the Vss was 0.378 L/kg. Tissue Pt distribution was similar for both Pt analogs with the greatest accumulation occurring in the kidney.
Conclusions: Cisplatin was well tolerated while mild alimentary tract signs were seen with carboplatin. Plasma Pt concentrations with cisplatin were similar to those of carboplatin and to those measured during treatment of solid tumors in human patients. Filterable plasma Pt concentrations for carboplatin persisted longer than for cisplatin, due mainly to the difference in Cl. Despite anatomical, physiological and biochemical differences among animal species, the PK disposition of Pt in cockatoos shares some features with the kinetics reported previously in rodents, dogs and human beings.

Rasagiline, a Selective Suicide Inhibitor of Monoamine Oxidase B, Increases Striatal Extracellular Fluid Dopamine Levels and Locomotor Stimulation Following L-dopa Without a Corresponding Increase in Dyskinetic Movements in a Parkinsonian Rat Model.

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Background: Rasagiline is a new selective monoamine oxidase B (MAO-B) inhibitor used in the treatment of Parkinson's disease alone or together with L-dopa. In rat brain tissue, the IC50 for inhibition of MAO-B *in vitro* is 4.4 ± 0.92 nM, with a selectivity ratio for inhibition of MAO-B:MAO-A of 1:94. Following oral administration to rats, the selectivity ratio for MAO-B:MAO-A inhibition *ex vivo* is 1:65 (based on ED50 values). In the striatum of rats with extensive unilateral nigro-striatal lesions induced by local injection of 6-hydroxydopamine (6-OHDA), dopamine (DA) behaves as a MAO-A substrate *in vivo*, however when the DA lesion is combined with a serotonergic lesion, inhibition of MAO-B produces an increase in extracellular levels of L-dopa-derived DA (DAec).
Methods: Rats were lesioned by injection of 6-OHDA to the left medial forebrain bundle (single lesion, SL), or by 6-OHDA together with 5,7-dihydroxytryptamine intracerebroventricularly (double lesion, DL). Rats were screened for DA lesion extent with apomorphine and then, commencing 3 weeks after the apomorphine test, were treated daily with rasagiline (0.05mg/kg s.c.) or saline. On the 14th day, microdialysates were collected from the striatum following L-dopa/carbidopa injection (25/6 mg/kg i.p.) and assayed by EC-HPLC. Another group of lesioned rats received L-dopa/carbidopa for two weeks daily with rasagiline or saline and the rotational behavior and dyskinetic movements following L-dopa i.p. were measured every two days.
Results: Rasagiline increased maximal DAec to a greater extent in DL than in SL rats (by 2.4 and 1.4 fold respectively) and with greater duration. In the DL rats, rasagiline treatment also significantly prolonged the duration of L-dopa-induced turning (T50 for turning duration = 131 ± 13.4 vs 91 ± 3.4 min, $P<0.05$), without a significant increase in total dyskinetic movement score (24.2 ± 12 vs 24.2 ± 11); n=5 for each treatment.
Conclusion:
1) MAO-B inhibition becomes important in the maintenance of L-dopa-derived DA levels in the striatum following combined DA and 5-HT denervation.
2) Locomotion is related to striatal DAec but dyskinesia is dependent on additional factors other than striatal DA release.

Recombinant Immunotoxins for the Treatment of Cancer.

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Background: recombinant immunotoxins are single chain antibodies genetically fused to protein toxins. The design of these agents dictates that the antibody portion binds a surface antigen on cancer cells and the toxin portion kills the targeted cells. Immunotoxins composed of a truncated version of Pseudomonas exotoxin (PE38) have been evaluated as therapeutic agents in clinical trials targeted to antigens on both hematologic and non-hematologic tumors.
Methods: immunotoxins were expressed in E coli. Clinical trials were conducted with IRB and FDA approval. For tissue culture experiments, cell lines were grown at low or high density and evaluated for immunotoxin killing using WST1 or assays for apoptosis.
Results: The best clinical results were achieved with the immunotoxin (BL22) targeted to CD22 on Hairy Cell Leukemia cells, where a 65% complete remission rate was reported. In contrast, targeting to mesothelin, an antigen expressed on mesotheliomas and ovarian cancers with the immunotoxin, SS1P, produced some partial responses and stabilization but no complete remissions. Comparing the two treatments reveals several differences: Hairy Cell Leukemia patients rarely make anti-PE38 antibodies in the first cycles of treatment (allowing retreatments) whereas ~90% of patients treated with SS1P do, (precluding retreatments). Also, leukemic cells are readily accessible to immunotoxin whereas target cells within solid tumors (such as ovarian and mesotheliomas) are presumably ~~more~~ difficult to reach. Other factors have also been investigated to explain the differential response, including shedding of target antigens and the possibility that tumor cells growing at high density are resistant to killing by immunotoxins. We have investigated the effect of cell density and found that a number of cancer cell lines exhibit a density-dependent resistance to killing by immunotoxins. Resistance was not due to failure of toxin delivery to the cell cytosol, as cells grown at high or low density were equally affected by a reduction in protein synthesis (PE38 inhibits protein synthesis). The basis for high-density resistance is currently being characterized.
Conclusions: Solid tumors are difficult to treat with immunotoxins. The reasons for this include poor access to tumor cells within tumor masses and possibly a novel resistance mechanism related to high density growth.

Jasmonates Kill Cancer Cells Selectively by Dissociating Hexokinase from Mitochondrial VDAC

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Background: Cellular bio-energetic metabolism and mitochondria are recognized as potential targets for anticancer agents, due to the numerous relevant peculiarities cancer cells exhibit. Jasmonates are anticancer agents that interact directly with mitochondria. Many types of cancer cells exhibit overexpression of the key glycolytic enzyme, hexokinase, and its excessive binding to mitochondria. These characteristics are considered to play a pivotal role in cancer cell growth rate and survival. The aim of this study was to identify mitochondrial molecular targets of jasmonates.
Methods: Binding and detachment of hexokinase from mitochondria were determined by hexokinase immunochemical and activity determinations, surface plasmon resonance analysis and planar lipid bilayer voltage dependent anion channel (VDAC)-activity analysis. Hexokinase expression was modified using hexokinase-overexpressing transfectants and its mitochondrial association.
Results:. We report that jasmonates bind to hexokinase and detach it from the mitochondria and its mitochondrial anchor— VDAC. Jasmonate-induced detachment from mitochondria occurs in various types of cancer cells including leukemia and solid tumors. Furthermore, the susceptibility of cancer cells and mitochondria to jasmonates is dependent on the expression of hexokinase, supporting a cause and effect relationship between jasmonate-induced hexokinase detachment and cell death.
Conclusions: 1) Our findings provide an explanation for the selective effects of jasmonates on cancer cells. 2) This is the first demonstration of a cytotoxic mechanism based on direct interaction between an anticancer agent and hexokinase. 3) The proposed mechanism can serve to guide development of a novel class of small anticancer compounds that kill cancer cells selectively by inhibiting the hexokinase–VDAC interaction.

Pharmacokinetics and pharmacodynamics of meloxicam in rats and humans

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Background: Meloxicam is a drug endowed of analgesic and anti-inflammatory activities. Although it is widely used in therapeutics, limited information concerning the pharmacokinetic-pharmacodynamic relationship is available. Aims of this study were to establish pharmacokinetic-pharmacodynamic modeling for antihyperalgesic and anti-inflammatory effects of meloxicam in the rat and to evaluate if levels are comparable to those reached in humans after administration of therapeutic doses.
Methods: Pharmacokinetic and pharmacodynamic evaluations were carried out in groups of rats using the paw thermal hyperalgesia and inflammation of the paw models. Measurements of effect and concentration of meloxicam were carried out at selected times for 6 hours. On the other hand oral pharmacokinetics of meloxicam in humans was evaluated in 24 healthy volunteers after an oral 7.5 mg dose and plasma concentrations were measured at selected times during 24 hours. Pharmacokinetic parameters were obtained by non-compartmental approach and effects against blood concentrations were fitted to the sigmoidal Emax model.
Results: Antihyperalgesic and anti-inflammatory effects against blood concentrations of meloxicam were fitted to the sigmoidal Emax model. EC50 for antihyperalgesic and anti-inflammatory effects were 15.1 ± 2.5 µg/ml and 10.01 ± 1.18 µg/ml, respectively; and Emax was in both cases about 75%. On the other hand, pharmacokinetic parameters obtained in humans were: C_{max} 0.70 ± 0.03 µg/ml, t_{max} 4.8 ± 0.65 h, AUC 27.7 ± 1.48 µg.h/ml and $t_{1/2}$ 24.5 ± 1.2 h.
Conclusions: A direct relationship between concentration and antihyperalgesic and anti-inflammatory activities of meloxicam was found, but the effect in rats is produced at much higher levels than the reached in humans at therapeutic doses.

Association between Polymorphisms of Vitamin D Metabolizing Enzymes and Colorectal Cancer Incidence

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Background: Vitamin D ($1,25(OH)_2D_3$) plasma levels are inversely associated with colorectal cancer incidence. Vitamin D levels are modified by the metabolizing enzymes 1,25-dihydroxyvitamin D3 24-hydroxylase (CYP24A1), vitamin D3 25-hydroxylase (CYP27A1) and 25-hydroxyvitamin D3-1-alpha-hydroxylase (CYP27B1) and by the vitamin D receptor (VDR). The association between polymorphisms of these vitamin D metabolism-related genes and colorectal cancer incidence has been investigated in a German population.
Methods: 18 common single nucleotide polymorphisms (SNPs) of CYP24A1, CYP27A1, CYP27B1 and VDR were determined in a series of 928 caucasian subjects (452 colorectal cancer patients and 508 healthy controls) from Germany using SNPlex™ technology (Applied Biosystems), PCR/RFLP, sequencing and GeneScan® genotyping assays. Allele frequencies and complex genoytpes were determined. Haplotype analysis (Phase 2.0) was applied to the received genotypes. Linkage disequilibria were analyzed with Haploview 3.32.
Results: SNP frequencies correspond to previous studies in Caucasian populations. Allele frequencies were without any characteristic variations regarding the case-control-classification. The polyA microsatellite analysis revealed a bimodal distribution of the alleles. No variant showed any significant deviation from the Hardy-Weinberg equilibrium. CDX-2 was found differently distributed between cases and controls (p=0.037). However, this association and a trend for CYP27B1_-1077 were no longer significant after adjusting for multiple testing by Bonferroni correction. Haplotype analysis and tests of complex genotypes and pairs of variants revealed no significant differences between cases and controls. We found a strong linkage disequilibrium between A2978T, PolyA, C1905A, TaqI, Tru9I and BsmI of VDR and between all three CYP27B1 variants (p<0.001).
Conclusions: The results of this study contribute to our knowledge about the relation between vitamin D metabolism-related genes and colorectal cancer incidence in Caucasians.

5-HT2a antagonists as a new treatment for JCV-associated progressive multifocal leukoencephalopathy

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Progressive multifocal leukoencephalopathy (PML) was first described as a complication of immune suppression fifty years ago. The prognosis has remained dismal since then, with discouraging results from clinical trials of various therapeutic approaches. PML is caused by reactivation of latent JC virus, and 5-HT_2a receptors have been identified as the main entry point for JC virus into glial cells. Since then, encouraging case reports have suggested several 5-HT_2a inhibitors (currently approved for other indications) may be effective in the treatment of PML. We discuss here preclinical data and perform a systematic review of case reports.

The study of the adhesion molecules in Non Small Cell Lung Cancer (NSCLC) treated with epidermal growth factor (EGF) can generate a new drugs targets and developing new approaches for systemic treatment in lung cancer

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Background: Lung cancer is the leading cause of cancer death in developed countries. At least, six important alterations were catalogued in defined molecular events that are common to all malignant cells: Recently, several investigators attributed some six events to an important group of molecules that are important component for adherence junctions between epithelial cells (adhesion cell-cell) called cadherins and other group which is responsible for adhesion of epithelial cells to extra matrix protein (ECM) called integrins. The purpose of this study was to assess the interactions of cell adhesion molecules (CAM) in cell lines from lung cancer, where 2 of these cell lines were non-metastatic (H-358, H441) and the other two which were metastatic cells (H1299, H292).
Methods: H358 bronchioalveolar cells, H441 lung adenocarcinoma cells, H1299 and H292 lung carcinoma cells were maintained in RPMI 1640 modified medium. The cell lines were treated with epidermal growth factor (EGF) for 30 minutes. Extraction of proteins from cultured cells was performed with denaturing buffer. Protein immunodetection was done by eletrophoretic transfer of SDS-PAGE, separation of proteins on nitrocellulose, incubation with antibody, and chemiluminescent second-step detection. In total of 20 proteins were performed from adhesion cell-cell and cell-extra matrix cellular (cadherins and integrins pathways).
Results: The results of cell-cell adhesion were not influenced by the treatment with EGF for 30 min. However we verified differences between the description types of cells. Otherwise, EGF could modulate the signaling pathways of the integrins and it we have show when different NSCLC cell lines are treated with EGF. We also observed antagonist functions between these proteins (PYK2 and FAK).
Conclusion: 1) The expressions patterns of adhesion cell-cell were not affect by EGF treatment, 2) The treatment can affect and may modulated the adhesion cell-ECM. 3) The antagonist effects can explain the crosstalk between EGFR and integrins pathways. Some proteins searched in this study may be a key of methastic, circulation and proliferation process in lung cancer and this may use to improve the therapeutical approaching of the NSLC.

Pain Relief Without Side Effects: Methylnaltrexone (MNTX) and Alvimopan (ALV) are Silver Bullets In the Use of Opioids for Analgesia

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Background: In 1978 Leon Goldberg noted that drugs acting on the opioid receptors of the gut and not crossing into the brain were available. Could we find an analogous opioid receptor antagonist?
Russell et. al (1982) first reported that the gastrointestinal (GI) effects of the morphine (MSO4) could be prevented by MNTX without affecting analgesia and this was later confirmed in volunteers and subjects on chronic opioids. Subjects given placebo (pbo) had transit of 104 min. MSO4/pbo increased transit to 163 min and MSO4/MNTX saw a return to 106 min, with no effect on analgesia.
ALV, while chemically distinct from MNTX, has a similar profile.
Efficacy: MNTX is approved for the treatment of opioid-induced constipation (OIC) in patients with advanced medical illness (AMI). Thomas et al. (2008) presented a RCT of MNTX administered 0.15 mg/kg, or pbo every other day for 2 wk in adults who were receiving opioids and had OIC in spite of laxative use. MNTX significantly increased laxation (48% vs. 15%), both through the 2-wk period and an extension.
ALV is approved for the management of post-operative ileus (POI). In a phase 2 RCT, patients with abdominal surgery given 6-mg capsule of ALV 2 hr before surgery and twice daily post-op had a significantly shorter median time to their first bowel movement (BM) vs. pbo (70 vs. 111 hours).
In bowel resection (BR) or abdominal hysterectomy (TAH), ALV significantly decreased the number of nasogastric tube insertions, time to GI recovery and time to hospital discharge (DC). Results were better with 12 mg than with 6 mg, and better in BR than in TAH.
Safety: MNTX in chronic opioid users and ALV in p-op trials show no reversal of analgesia or change in opioid use.
MNTX is associated with abdominal cramping, gas and discomfort. Orthostatic hypotension in volunteers was dose limiting in volunteers.
In clinical trials of POI ALV has shown a similar side-effect profile to pbo. Trials of ALV for chronic use in opioid-induced bowel dysfunction (OBD) were briefly on hold due to non-statistically higher rates of cardiovascular events, some malignancies and bone fractures, but have been restarted.
Higher doses of MNTX or ALV in tolerant subjects, while not inducing classical opioid withdrawal, will produce GI distress.
Conclusions: MNTX and ALV are new tools for the management of the side effects associated with opioids.

Authors' disclosure statement:
Dr. Foss is a patent holder for MNTX and receives royalties from the sales of the drug.

Omalizumab: The First Real Magic Bullet for the Treatment of Allergic Asthma?

FOX H

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Severe, uncontrolled asthma can be fatal and is associated with high levels of hospitalization and urgent care visits. Moreover, it has been estimated that up to 90% of asthma cases have an allergic component. Allergen exposure causes B cells to differentiate into plasma cells that produce and release immunoglobulin E (IgE) antibodies into the circulation. IgE then binds to high-affinity IgE receptors (FcεRI) on the surface of tissue mast cells or peripheral-blood basophils that, in the presence of allergen, results in rapid release of inflammatory mediators. These inflammatory molecules can result in bronchoconstriction leading to an asthma exacerbation. Moreover, there is a strong correlation between FcεRI receptor expression and fatal asthma. As such, there is a clear rationale for the development of asthma treatments that target IgE.

Omalizumab is a humanized monoclonal antibody that inhibits the binding of IgE to the FcεRI receptor. Reduction in surface-bound IgE on FcεRI-bearing cells limits the release of allergic response mediators. Omalizumab also reduces the number of FcεRI receptors on basophils in atopic patients. In patients with severe persistent allergic asthma that remains inadequately controlled despite available therapy, the addition of omalizumab leads to significant reductions in the incidence of clinically significant asthma exacerbations and emergency visit rates compared with placebo. Furthermore, omalizumab significantly improves asthma-related quality of life, morning peak expiratory flow and asthma symptom scores compared with placebo. Reduction in free IgE levels correlates well with improvements in clinical outcomes achieved with omalizumab. Following cessation of omalizumab, IgE returns to baseline levels and, after a small delay, signs and symptoms of asthma resume. Observed significant reductions in eosinophil counts compared with placebo support the anti-inflammatory effects of omalizumab. Real-life experience confirms the therapeutic benefits of omalizumab observed in clinical studies.

A targeted anti-IgE approach to therapy with omalizumab has revolutionized the treatment of severe persistent allergic asthma and vastly improved clinical outcomes in some patients (≥12 years old). Ongoing research aims to identify the full potential of this magic bullet in IgE-mediated disease, including paediatric allergic asthma.

Ixostatin, a Novel Tick Salivary Protein that Specifically Binds to the Somatomedin B Domain of Vitronectin and Prevents its Interaction with Integrin alphavbeta3 and Urokinase Receptor

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Saliva from blood-sucking arthropods is a rich source of modulators of vascular biology. In this report we describe the cloning, expression and mechanism of action of Ixostatin, a novel family of 10 kDa cysteine-rich peptide from Ixodes scapularis salivary gland. Recombinant Ixostatin was expressed in insect cells and was produced in an active form. Surface plasmon resonance experiments show that Ixostatin interacts with monomeric or multimeric human vitronectin with a KD ~ 0.5 nM, but does not bind to other extracellular matrix proteins. Notably, the high-affinity binding site for Ixostatin was identified as the somatomedin B domain (SMTB) of vitronectin. In addition, Ixostatin at nanomolar concentrations inhibits integrin alphavbeta3- and urokinase receptor-mediated cell adhesion to vitronectin, but display negligible effects in fibrinolysis in vitro. It is concluded that the most prominent biological property of Ixostatin is to negatively modulate cell adhesion to the extracellular matrix. Ixostatin is the first ligand from an exogenous source that specifically targets the SMTB domain of vitronectin. It also represents a novel mechanism by which tick saliva manipulates vector-host interactions, and may therefore have potential medical applications.

Are the tropical forests the new frontier for antibiotics discovery? Novel peptidomics insights on the screening of plant antimicrobial peptides.

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Background: During human history, microbial infections directly affect World population in several areas causing economical, social, agricultural and health problems. This situation has only been controlled with the development of antibiotics. Nevertheless, the inadequate use of available compounds leaded to microorganism's resistance. In order to control this problem, different sources of antimicrobial peptides (AMPs) have been screened, including plants, microorganisms, amphibian, sea animals and several others. These small peptides show different and special abilities. They are able to inhibit digestive enzymes or act against bacteria and/or fungi.
Methods: Once those common sources have been deeply explored, in this report AMPs were screened from flowers and fruits collected from Brazilian tropical forest. In both cases were used a combo of classical strategies, which included HPLC chromatography's and bioassays against human pathogens such as *Klebsiella* sp., *Proteus* sp. and *Aspergillus fumigatus*. All MICs were calculated. Futhermore novel peptidomics strategies such as liquid isoelectric focusing (pI 3-11) associated to LC-MS techniques were also utilized in order to identify antimicrobial peptides in large scale.
Results: Flowers and fruits showed AMPs from novel classes with different structures, evaluated by molecular modeling and dynamics. Among them was observed glycine-rich peptides and peptides pertaining to unpublished classes. Moreover, all of them were able to cause a remarkable reduction on gram-negative and gram-positive bacteria. Additionally, peptidomics techniques showed that it was possible to identify, by liquid IEF followed by LC-MS, at least 30 different peptides with similar properties to antimicrobial peptides, such as cationic and hydrophobic peptides.
Conclusions: Current research in this area here focused, particularly aims to control pathogenic microorganisms, showing that antimicrobial peptides could be extracted and further commercialized in a near future as a common drug. Furthermore, this research also shows that tropical forests culd provide new classes of antimicrobial peptides, helping to solve the infections problem.

Limitations of Non-magic Bullets Compounds in Bioequivalence Assessment. How Can this Enhance Knowledge Towards the Development of Generic Products. The Mycophenolate Mofetil Case.

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Background: Immunosuppressive drugs such as mycophenolate mofetil have been used as prophylaxis of acute transplant rejection in patients receiving allogeneic renal, cardiac or hepatic transplants. Undesirable effects oppose to its clinical efficacy and therein detach it from the magic bullet concept, when seen in clinical and therapeutic perspectives only. When looking into its pharmacokinetic profiling mycophenolate mofetil fits into a standard bioequivalence approach where no major obstacles are foreseen in the clinical trial design. Aims: 1) To compare the rate and extent of absorption of mycophenolate mofetil 500 mg tablet versus reference product. 2) To optimize clinical trial design.
Methods: This study included 12 healthy adult male and female volunteers to whom test and reference formulations were administered as a single-dose, 1x500 mg tablet, in a randomized, open-label, 2-way crossover fasting design. Blood samples were collected until 12.0 hours post-dose and up to 48.00 (±0.5) hours post-dose for mycophenolate mofetil and mycophenolic acid, respectively. Pharmacokinetic parameters AUC_{0-t}, AUC_{0-inf}, C_{max}, residual area, T_{max}, $T_{1/2el}$ and K_{el} were assessed following statistical analysis: parametric ANOVA on AUC_{0-t}, AUC_{0-inf}, C_{max}, geometric confidence intervals (CI) for AUC_{0-t}, AUC_{0-inf}, C_{max} and non-parametric test (Wilcoxon) for T_{max}. Ln-transformed bioequivalence standard parameters, AUC_{0-t}, AUC_{0-inf}, C_{max} were looked into bearing in mind the 80% to 125% acceptance criteria.
Results: Mycophenolate mofetil AUC_{0-t}, AUC_{0-inf}, C_{max} intra-subject coefficient of variation were 23.32%, 24.19% and 49.03% and mycophenolic acid's 6.16%, 5.23% and 48.33%, respectively.
Conclusions: 1) High unexpected intra-subject CV for bioequivalence parameters may imply sample size readjustment for a fullscale study and design fit. 2-way crossover design may not be the adequate. 2) Regulatory standpoint for highly variable drugs urges to best optimize bioequivalence process.

Population Pharmacokinetics for Nevirapine in Black Newborns to Prevent HIV Transmission

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Background: A prevention of mother-to-child transmission program to reduce risk of HIV transmission was carried out in Uganda. 62 HIV-positiv pregnant women and their newborns participated. Nevirapine (NVP), a non-nucleoside reverse transcriptase inhibitor, was administered as single oral dose to all participants. Population pharmacokinetic (PK) models for mothers and newborns were developed to describe NVP concentration-time profiles for different individuals.
Methods: After a single oral 200 mg dosing to mothers during labour and a 2 mg/kg NVP dosage to newborns after birth, 113 plasma, 95 breast milk samples of mothers and 113 plasma samples of newborns were available. Population PK analysis for mother and newborn data were performed using the nonlinear-mixed-effect modeling approach implemented in NONMEM™ (ADVAN6, TOL5; FOCE INTERACTION estimation method). Final PK models were used for simulating entire concentration-time profiles for different percentiles (P_{5-95}) of individual PK parameter distribution.
Results: Due to sparse data, absorption rate constant was fixed to 1.66 h^{-1} [1]. A 2 compartment PK model was developed for mother plasma and milk data. V/F (105.4 L) and CL/F (1.5 L/h) resulted in a long half-life of 49 h. Intercompartmental clearance was high (115 L/h). Interindividual variability (IIV) was implemented in CL1/CL2 (28% CV). For newborn data a PK model with 'multiple input' was developed. Different input routes from mothers were combined in a 'bioavailability' factor (18%). Plasma/placenta-plasma/milk transfer rate constant, V2/F and CL/F were estimated to be 5.1 h^{-1}, 25.8 L and 0.30 L/h, respectively, resulting in a half-life of 61 h. IIV was implemented in F' (59% CV) and V2/F (29% CV). Simulated concentration-time profiles revealed long-term exposures for mothers and newborns with NVP >IC_{90} for 10-24 d and 12-22 d for different indivduals, respectively.
Conclusions: Population PK models for mother and newborn data were successfully developed to guide single dose NVP prevention strategies of HIV transmission from mother-to-child.
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Novel BINOL Derivatives as Photoactivatable Carriers of DNA-Targeted Potent Cytotoxic Agents

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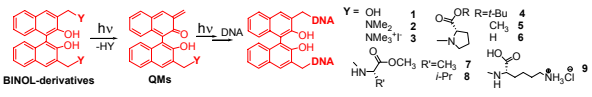
Background: Several strategies have been developed for a selective and mild activation of DNA-modifying agents, but limited examples of photochemically activated DNA-alkylating agents have been reported. In fact, to date, psoralens are the only well-established class of drugs known to induce DNA or RNA cross-linking upon photoactivation. Recently, our research has been focused on another class of reagents that similarly express a triggerable ability to alkylate DNA.
Methods: This study included: 1) synthesis of a small library of BINOL-derivatives (**3**-**9**); 2) mechanistic insights from time-dependent product distribution analysis and laser flash photolysis (LFP); 3) evaluation of the DNA cross-linking by compounds **3**-**9**, using a super coiled plasmid DNA (pBR322) in an alkaline agarose gel assay; 4) DNA alkylation in the cellular environment, using LoVo cells performed by alkaline comet assay.
Results: Photoactivation at 360-450 nm of the BINOL-derivatives **1**-**9** yielded a short-lived, high energy intermediate undergoing alkylation and DNA cross-linking with high photoefficiency and superior cytotoxicity. Detection of the transient, by laser flash photolysis (LFP), suggests that BINOL-quinone methides (QMs) are key intermediates in the process. QMs trapping experiments, monitored in a time-dependent product distribution analysis, demonstrated that the phototriggered reactivity of these BINOL-derivatives as bis-alkylating agents is the result of a two-steps process involving sequential generation of monoalkylating QMs. Light activation of the BINOL-L-amino esters produced cytotoxic QMs very effective against human tumor LoVo cells with EC50 in the 130–230 nM range.



Conclusions: Trimethylpsoralen (PS) is about 4 times less potent than our newly tested compounds. The BINOL-L-proline methyl ester (**5**) showed notable photoselectivity, since it displayed cytotoxic effects upon irradiation only and was able to efficiently reach the target DNA inside the cells, where it form both alkylated and cross-linked adducts.

Circular dichroism spectroscopy in stereochemical studies of β-lactam antibiotic analogues

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Since the introduction of penicillin onto the market in 1940, the β-lactam antibiotics are amongst the most frequently prescribed pharmaceutical products to cure many diseases. However, the extensive use of penicillins and cephalosporins has created an increasing number of resistant strains of bacteria. The continuing growth of the bacterial resistance has prompted the search for new structural variants of β-lactam antibiotics with enhanced and/or novel biological profiles. Clavams and oxacephams, representing oxaanalogues of penicillins and cephalosporins, respectively, have demonstrated that the high biological activity of β-lactam antibiotics is not dependent on the presence of the sulfur atom. Some oxa- and carbaanalogs are more active than natural congeners and exhibit high activity in both of the enantiomeric forms.[1] In this context, significant biological activity of β-lactam derivatives, closely related to the stereostructure, calls for methods which allow an unequivocal and reliable determination of an absolute configuration. Circular dichroism spectroscopy has been used successfully for this purpose, and it appears to be a convenient, sensitive and fast technique for the stereochemical assignment of azetidinones and their polycyclic derivatives.[2]
In view of the foregoing the relationship of chiroptical properties and molecular structures of penicillins, cephalosporins in respect of their oxa- and carbaanalogues together with of monobactams will be examined. The applicability of the helicity rule, previously established by us,[2] which correlates structure to CD spectra in respect of all classis of β-lactam derivatives will be tested. It will be demonstrated that the rule obeys a variety of oxacephams, clavams and their carbaanalogues.
Despite the appealing simplicity and the apparent success of the helicity rule, the underlying assumptions of conformational rigidity and electronic decoupling of the amide chromophore require further validation. Therefore, for the representative β-lactam derivatives the ECD spectra computed by means of time-dependent density functional theory (TDDFT)[3] will be compared to the experimental curves. These model compounds cover the therapeutically important classes of cephams, oxacephams, carbacephams, and their non-classical analogues. The obtained results pointed to a surprisingly high sensitivity of the CD to the molecular conformation. Nevertheless, the helicity rule may provide a good first guess of the absolute configuration of cepham analogues. For a more definite assignment of the absolute configuration, however, the good corroboration of the predictions made by the helicity rule by the computational results is recommended.[3]

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Formulation Challenges in Biologics

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Background: Biotechnology derived products have reached a share of more than 30% of the newly approved drugs. The formulation of these compounds presents specific challenges. This is due to the parenteral application and specific instability. Additional challenges are presented by antibodies which often require high dosing.
Methods: Various routes of formulation of highly concentrated antibody formulations have been tested. The pros and cons of different manufacturing methods are discussed. As a second example the formulation of hydrophobic cytokine is presented which demonstrates solutions to problems with adsorption and protein-protein interactions.
Results: Ultrafiltration / Diafiltration processes like tangential flow filtration are suitable but the flow rate is reduced with increased protein concentration and viscosity. At the same time substantial shear stress can affect the protein structure. As an alternative drying via lyophilisation or spray drying followed by reconstitution with less liquid is a favoured approaches. But this process leads to enrichment of excipients next to the protein which has to be considered. Furthermore reconstitution times can be substantially increased. Precipitation and crystallisation of antibodies followed by application of a suspension or a solution after redissolution may be limited by protein stability and residual precipitation agent. Overall physical stability of antibodies may be scrutinized due to the high concentrations leading to aggregation and precipitation. Syringeability has to be assured despite the increased viscosity with protein concentrations and can be controlled e.g. by pH and ionic strength. For the formulation of hydrophobic proteins, adsorption phenomena can be resolved by adjusting pH and ionic strength in combination with surfactants and the container quality. HSA as an excipient substantially changes its properties depending on the formulation and may not be adequate for stabilization of protein drugs.
Conclusions: (1) high concentration liquid formulations can be realized by drying-, TFF- and precipitation - processes; (2) go for HSA-free formulations (3) formulation development of biosimilars requires an even better understanding (4) develop appropriate analytic tools e.g. FTIR, fluorimetry.

Psoriasis [Ps]: A Possinle Candidate for Vaccination

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Background Ps is a common skin disease affecting 2-3% of the European and American populations. It is a mutifactorial disease with both genetic and enviromental factors. Histlogically Ps is characterised by hyperproliferation of the keratinocytes. It was shown some 20 years ago that this hyperproliferation was T cell mediated However what has eluded detection is the antigen responsible for initiating this immune response.
Methods. Clinical evidence has shown that psoriasis can be triggered by beta hemolytic streptococci [BHS]..Our group has studied T cell responses to BHS in the blood and skin.
Results. T cells in the blood and skin responded to BHS extracts. Next we showed that this reponse was triggered by cell wall extracts. Further studies have now shown that the cell wall antgen was peptidoglycan [PG] and this is an adaptive immune response
Conclusions. PG is also known to trigger the innate immune response and this system is known to be involved in the pathogenesis of Ps. The antigen in psoriasis may therefore be the peptide brides in streptococcal PG. The identification of this peptide opens the way to possible vaccines for Ps.

Estrogen Receptor Beta may be a Novel Target for the Beneficial Effects of Estrogens in Females, and Androgens in Males, for Anxiety, Depression, and Cognitive Function

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Background: Hormone-replacement therapies may have some beneficial effects for cognitive or affective processes; however, their effects and mechanisms in this regard are not well-characterized. Studies in our laboratory have focused on the effects and mechanisms of estrogens, such as 17β-estradiol (E_2), and androgens, such as the 5α-reduced metabolite of testosterone, 3α-androstanediol (3α-diol), for their anti-anxiety, anti-depressant-like, and cognitive-enhancing effects in female and male rodents. Moreover, estrogens have well-known trophic effects, which can increase risk of some cancers. As such, it is important to discern the mechanisms of steroids for their beneficial versus unwanted proliferative effects. Our laboratory has been investigating the β isoform of the estrogen receptor (ER) as a putative target for these effects.
Methods: We have investigated effects on anxiety/depression and cognitive behavior utilizing the following three approaches. First, the effects of systemic or intra-brain administration of selective ER modulators (SERMs) or selective androgen receptor modulators (SARMs), which vary in their affinity for ERα or ERβ, to female and male rodents were assessed. Second, intra-brain administration of ERα and/or ERβ antisense oligonucleotides (AS-ODNS) to rats administered SERMs or SARMs was utilized. Third, the effects of SERMs or SARMs administration to mice with targeted deletions of ERβ (βERKO) were assessed. Furthermore, we have determined the whether some of these treatments alter tumorigenesis following exposure to a chemical carcinogen.
Results: Systemic or intra-brain administration of SERMs or SARMs reduces anxiety and depression-like behavior of rats and mice. ERβ, but not ERα, AS-ODNs attenuate the beneficial effects of E_2 and 3α-diol. Effects of SERMs or SARMs to reduce anxiety-like behavior and enhance cognition are not observed in βERKO mice. We have preliminary evidence about the tumorigenic role of SERMs, and have shown that E_2 increases tumor burden of young ovariectomized rats.
Conclusions: These data support the notion that the beneficial effects of estrogens in females or androgens in males for psychological (improve affect/mood, cognition) may be via actions at ERβ.

Role of antiproliferative enzyme indoleamine (2,3)-dioxygenase in the impaired immune function in infectious diseases

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In several pathologic conditions like infections, autoimmune syndromes, cardiovascular and neurodegenerative disorders as well as malignant disease, activation and inflammation are strongly involved. Pro-inflammatory cytokines like interferon-γ (IFN-γ) play a dominant role in the clearance of infections with viruses or intracellular bacteria and parasites but also in the development of inflammation. In various cells, the expression of tryptophan-degrading enzyme indoleamine (2,3)-dioxygenase (IDO) is induced by IFN-γ as a part of its antimicrobial armature. Activation of IDO restricts protein biosynthesis by deprivation of essential amino acid tryptophan and thereby growth of pathogens is halted. As a side effect, also development and proliferation of normal host cells like activated T-lymphocytes is diminished. Accordingly, IDO appears to represent a critical step within host-response directing whether immune activation is successful in controlling an intracellular infection or whether T-cell responsiveness is hampered, and consequently a persistent infection is developing.
Increased degradation of tryptophan has been described, e.g., in patients with HIV infection, in Streptococcus pyogenes infection as well as in Lyme neuroborreliosis. Tryptophan deprivation as a result of the microbicidal activity of IFN-γ appears to be involved also in the pathogenesis of anemia when erythroid progenitor cells suffer from insufficient tryptophan supply. Also weight loss and cachexia are closely linked to inflammatory response when protein biosynthesis of the organism is restricted by diminished tryptophan availability. In the absence of any ability to synthesize tryptophan, upon shortage of tryptophan cells begin to degrade protein to sequester tryptophan for production of highly needed proteins. Finally, tryptophan shortage affects biosynthesis of neurotransmitter 5-hydroxytryptamine (serotonin) and lead to the production of potentially neurotoxic tryptophan catabolites such as quinolinic acid. Both these biochemical cascades seem to be involved in the development of neuropsychiatric symptoms like cognitive impairment and depression especially in patients suffering from severe and chronic infections. Thus, accelerated tryptophan degradation by IFN-γ-induced IDO can give raise to an immune activation syndrome in patients suffering from infections, which is characterized by subnormal tryptophan levels and is associated with adverse outcome.

Application of pharmacokinetics of individualize antineoplastic therapy

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Background: Antineoplastic drugs usually are administered at the mean maximum tolerated dose in the patient population. However, these doses may result in considerable interindividual exposure differences because of individual pharmacokinetic properties. These differences explain a part of individual differences in toxicity and efficacy. Such variation occurs in systemic as well as in local pharmacokinetics.
Systemic pharmacokinetics in an individual may be assessed by therapeutic drug monitoring (TDM) but with the disadvantage that the information is available only after administration of the first dose. This however is useful only if there is a relationship between concentrations at selected time points and drug effects. For some drugs, key components of variability in systemic pharmacokinetics are known, such as activity of a metabolizing enzyme which may be related to an underlying genetic polymorphism, sex, or renal function. This enables a prospective dose adjustment without measuring individual concentrations. Important examples include the adjustment of doses to BSA, to renal function (carboplatin), and to *UGT1A1* (irinotecan) or thiopurine S-methyltransferase genotype (thioguanin). Indeed, a more extensive assessment of enzyme activity by phenotyping and/or genotyping has the potential to further decrease the unexplained fraction of interindividual variability.
In malignant disease, there are also pronounced differences in **local pharmacokinetics**, such as distribution of a drug into the tumor interstitium, which cannot be explained exclusively by tumor size and vascularization, as well as uptake and metabolism by the tumor cell. Local pharmacokinetics is difficult to assess. Microdialysis may be used to measure interstitial unbound drug concentrations but this tool is limited to experimental studies and cannot be applied to each patient. Biopsies are required to characterize intracellular pharmacokinetics. Thus, while occasionally CSF concentrations are measured to assess CNS exposure, currently local pharmacokinetics cannot be taken into account for treatment individualization.

Pharmacokinetics of Antimycotic Drugs during Continuous Renal Replacement Therapy

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Background: Critically ill patients frequently suffer from acute renal failure requiring continuous renal replacement therapy. The risk of fungal infections is significantly increased in these patients at the intensive care unit. Knowledge of the influence of renal replacement therapy on the elimination of antimycotic drugs is essential to provide adequate dosing. Elimination of any given drug by renal replacement therapy is influenced by membrane-specific factors, due to physico-chemical properties of the drug, and characteristics of the renal replacement therapy. We review available data concerning pharmacokinetics and dosing recommendations of antimycotic drugs during continuous renal replacement therapy.
Methods: Review of studies investigating the pharmacokinetics of antimycotic drugs during continuous renal replacement therapy.
Results: No dosage adaptation seems to be necessary in most antifungal drugs during continuous renal replacement therapy. This may be explained by their high protein binding, high molecular weight and mainly non-renal elimination. In contrast, fluconazole is mainly eliminated via the kidneys. However, as continuous renal replacement therapy results in fluconazole clearance similar to that of individuals with normal renal function, no dosage reduction is recommended. Voriconazole´s pharmacokinetics is barely affected during continuous renal replacement therapy. However, attention should be directed to its intravenous vehicle sulphobuthylether beta-cyclodextrin which may accumulate during renal replacement therapy. Switching to the oral route seems to be rationale as soon as sufficient gastrointestinal absorption is ensured in patients requiring treatment with voriconazole.
Conclusions:
1. Continuos renal replacement therapy does not influence the dosing regimen of most antimycotic drugs.
2. As fluconazole is effectively removed via continuous renal replacement therapy, no dosage reduction is required despite its mainly renal elimination.
3. Voriconazole should be administrated via the oral route when sufficient gastrointestinal absorption is ensured to avoid accumulation of the intravenous vehicle sulphobuthylether beta-cyclodextrin.

The VesiVax® System: Vaccinology's Magic Bullet?

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Recently, there has been significant emphasis placed on the development of vaccines that address the effectiveness, safety and manufacturing issues associated with the classical method of pathogen based vaccines. Towards this end, the *VesiVax®* vaccine system was designed, which employs a flexible and easily modified gene cassette designed to rapidly engineer and produce recombinant antigen proteins that are compatible with bilayer membranes. The recombinant antigen proteins consist of a target epitope or antigen fused to an aqueous soluble hydrophobic domain that makes purification simple yet allows for stable insertion of the immunogen within the lipid membrane. Immunogenic liposomes consisting of a well-defined set of lipid constituents incorporating the recombinant antigen protein can then be produced using industry standard manufacturing processes.

Vaccines based on the *VesiVax®*system have been constructed against several pathogens including the influenza virus and herpes simplex type 2 virus, the causative agent of genital herpes. These vaccines have been tested in animal models and have demonstrated significant protective efficacy from microbial challenge and have elicited strong immune responses. Assays of the immunological parameters suggest that both T and B cell responses can be elicited by *VesiVax®* vaccines. Taken together, the inherent flexibility of the *VesiVax®* platform is expected to facilitate the rapid development of new vaccines which are effective at stimulating protective immune responses..

Comparison of susceptibility against MRSA isolates to the brand vancomycin and manufactured generic drugs

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Background: The brand vancomycin for injection(VCM 1) was released for the treatment of the methicillin-resistant *Staphylococcus aureus* (MRSA) infection. At present, 11 generic vancomycin products are available in Japan. Of them, five have been available for several years already. The bulk of brand vancomycin is imported from the United States, and the products for injection are manufactured in Japan. However, most of the generics of vancomycin used in Japan are imported as finished product or by bulk from Taipei, France, Hungary, and Slovenia.
Aim & Method: The objective of this study was to test VCM 1 for its activity against 80 clinical isolates of MRSA and to compare the results with those obtained with generic products. We investigated the susceptibility of 80 MRSA strains to brand vancomycin (VCM 1) and 5 generic products (VCM 2-6). The antibacterial activity of VCM 1 and VCM 2-6 was determined using the CLSI broth microdilution method. Furthermore, we compared the potency equivalent per vial of VCM 1 and these 5 generic products by a bioassay.
Results: The MIC50 of VCM 6 was 1 mg/L, while that of VCM 1-5 was 0.5 mg/L. The MIC of generic VCM 6 was slightly behind in comparison with other vancomycin products. In this study, the potency equivalent of VCM 1 and generic VCM 6 was 495 mg/vial and 455 mg/vial, respectively. The potency equivalent of the generic products was slightly lower than that of VCM 1.
Conclusions: Although the potency equivalent of the VCM used in this study was within the range accepted by the United States and Japanese pharmacopeia, the results showed that the susceptibility of one generic product was not similar to VCM 1. Because vancomycin shows side effects such as nephrotoxicity, therapeutic drug monitoring using a pharmacokinetic parameter is performed for chemotherapy. The population parameter necessary for this analysis consists of data obtained using VCM 1. Therefore, it is possible that some generic products whose potency equivalent of vancomycin is lower than that of the brand drug will have a weak effect on the infectious agent.

Endovascular Stenting for Malignant Superior Vena Cava Syndrome is Essential Therapy but not Approved in Japan

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Malignant superior vena cava (SVC) syndrome is difficult problems and associated with poor outcome in patients with lung cancer. Radiotherapy and/or chemotherapy could once improve the condition. Subsequently, the tumor becomes refractory and SVC syndrome appear again, constructive therapy including stent placement is abandoned since the case is viewed as terminal and treatment becomes palliative. Moreover, vascular stenting is not approved in Japan. A 66-year-old women with advanced primary lung cancer (adenocarcinoma cT1N3M1 stage IV BRA OSS) treated with 4 cycles of irinotecan/carboplatin combination chemotherapy, two times of Gamma Knife radiosurgery for brain metastasis, and radiotherapy for bone metastasis with cancer pain. Ten months later, the patient treated with oral fluoropyrimidine anticancer drug S-1 for second line, and drainage against malignant pleural effusion. For third line therapy of gefitinib, the patient maintained stable condition for a while. One year and 7 months after the onset, the patient developed severe swelling of face and both arms as SVC syndrome. We recognized that is the timing to place a self-expandable metal stent in the SVC. However, in the treatment group discussion, we attached importance to that endovascular stenting is not approved in Japan, and decided to not use the stent. The patient underwent radiation therapy (48 Gy in 20 fractions) with irinotecan (40 mg/m2/week) chemotherapy. The symptoms of SVC syndrome were resolved once, and took a turn for the worse within the chemoradiotherapy. Finally, the patient died one year and 10 months after the disease onset with miserable watched severe swelling of the upper half of the body especially face. A *post mortem* examination showed complete response and almost no remaining tumor, but thrombus obstruct the SVC. Recent development of stent placement therapy for the treatment of malignant constriction has improved the quality of life, and possibly survival. In cases like our patient, chemoradiotherapy reach the limit for SVC syndrome, and Stenting is essential. The approval of endovascular stenting for SVC syndrome is warranted in the worldwide.

Chelating Agents for Treatment of Uranium-induced Toxicity in Radiation Emergency Medicine

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Backgroud: Radiation workers treating actinides in the nuclear fuel cycle are exposed to accidental internal contamination of uranium. One of route of uranium intake is via wounds. Chelation therapy is an optimal method for reducing uranium-induced toxicity. So far, we examined on the effects of catechol-3,6-bis(methyle-imino-diacetic acid) (CBMIDA) by local treatment in simulated wounds, in which the depths contaminated with uranium in wounds are different.
Methods: Male Wistar rats, 8 weeks old, were divided into three groups (n=42/group) of intracutaneous(IC), subcutaneous(SC), and intramuscular (IM) injection. After the injection of DU (4 mg/kg, pH 1) by the different routes, rats of each group were infused with 480 mg/kg (adjusted to pH 6.8 by bicarbonate, molar rate 78 times of uranium) of CBMIDA into the DU-injected site at 10, 30, 60, 120 min and 24 hours (7 rats at each point). Rats were killed 24 hours after CBMIDA treatment. Data obtained were compared with that in the corresponding no-treated group, respectively.
Results: When CBMIDA was administered within 10 - 60 or 120 min after DU-injection, the uranium concentration of the DU-injected site decreased significantly ($P<0.05$) to 3-29 % (IC), 4-11% (SC), and 25-32%(IM) of that in the no-treated group. Amounts of excreted uranium increased to 4-5 times. Uranium concentrations in the kidney, as the target organ of uranium, decreased to 35-74% (IC), 22-42% (SC), and 12-39% (IM). Regarding to the kidney, as the target organ, the improvement of dysfunction by serum and urinary examinations and tissue damages by histological observation, were confirmed. Also, CBMIDA improved not only the damage by chemical action of uranium but also the burn by acid solution in the DU injected site.
Conclusions: The results indicated that CBMIDA is the useful chelating agent, (1) to increase uranium and (2) prevent the tissue damages and dysfunctions of organ, in the treatment for the wounds contaminated accidentally with uranium, if CBMIDA applies as early as possible after the intake.

Trabectedin: a new anticancer bullet from the sea

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Background: Trabectedin (formerly ecteinascidin ET-743) is a potent antitumor tetrahydroisoquinoline alkaloid in clinical use originally derived from a marine tunicate and now obtained by synthetic modification of microbially produced cyanosafracin B.
Methods: A variety of physico-chemical techniques, including the use of fluorescently labelled oligonucleotides, have been employed to test the ability of trabectedin to bind covalently to DNA. Insights about the activation and binding mechanisms have been gained both experimentally (i.e. nuclear magnetic resonance spectroscopy) and computationally (i.e. molecular dynamics simulations). The panel of 60 human tumor cell lines of the National Cancer Institute (NCI) Anticancer Drug Screen was used to reveal a rather unique activity profile that encouraged further development as an anticancer agent. Additional work has been done on mammalian and yeast cells both proficient and deficient in several DNA repair mechanisms.
Results: A definite role for hydrogen bonding has been demonstrated for sequence recognition and binding orientation of trabectedin in the DNA minor groove. TGG, CGG, AGC, GGC, and AGA triplets have been identified as the preferred DNA sites for stable adduct formation with the central guanine. As a consequence of trabectedin bonding, the double helical structure is only minimally perturbed except for widening of the minor groove and a net smooth bending towards the major groove due to the introduction of positive roll. The close contacts that are established between trabectedin and both DNA strands give rise to a significant increment in the stability of the resulting drug-DNA complexes, which is thought to result in stalled replication and transcription forks that can lead to double-strand breaks. Cell sensitivity to trabectedin is dependent on the cellular status of proteins involved both in transcription-coupled nucleotide excision repair (TC-NER) and in homologous recombination (HR). Trabectedin is currently approved for the treatment of soft tissue sarcoma and is an orphan medicinal product for relapsed ovarian carcinoma. Toxicity to trabectedin is dose-related, mostly limited to bone marrow and liver, and follows a transient-reversible pattern.
Conclusions: 1) Trabectedin is a novel chemical entity endowed with a new mode of action, (2) Patients harboring tumour cells with proficient TC-NER and deficient HR systems would be expected to respond best to this drug.

Authors' disclosure statement:
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Perspective Chemotherapeutic Combination to Combat Flu

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Background: Previous studies of ours demonstrated a marked synergistic combination effect of rimantadine and oseltamivir in 100:1 compounds doses ratios in experimental infection with influenza A (H3N2) in mice when the treatment course onset was on the day of virus inoculation. Considering these data we studied combination effect of both compounds in 50:1 and 25:1 ratios in order to determine the dose ratios scope preserving a high efficacy. The antiviral effect of the treatment course with the combination started 24-hours after virus inoculation was tested.
Methods: White male mice 16-18 g were inoculated intranasally with 0.05 ml/mouse of influenza A/Aichi/2/68 (H3N2) virus. Rimantadine hydrochloride and oseltamivir phosphate were administered *per os* in five-day-treatment course beginning 4-hours before or 24 hours post-virus inoculation with 20 – 30 MLD_{50}. Protection index (PI) and mean survival time (MST) were determined through 14 days post infection. Infectious virus titers were determined in Madine-Darby canine kidney cells. Lung consolidation score and lung index were evaluated.
Results: Combinations of selected doses of 5, 10 and 20 mg/kg/day rimantadine and 0.1, 0.2, 0.4 and 0.8 mg/kg/day oseltamivir were combined in doses ratio 50:1. PI up to 82.7% and 91.3% and MST up to 13.2 and 13.6 days for certain combinations were evaluated, while the individual effects of the same doses were from 13.3% to 30.6% PI and 7.9 to 9.8 days MST, respectively. Determination of lung virus titers and lung parameters in combination-treated groups also proved the synergistic effect of both therapeutics.
Conclusions: Oseltamivir and rimantadine at daily doses up to 50 times lower than optimal effective one for oseltamivir and 8-16 times lower - for rimantadine in 1:50 ratio demonstrated synergistic effect when administered in combination in experimental infection with influenza virus A (H3N2) in mice.

Anti-leishmanial effect of Hydroxyurea

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Background: Protozoa are eukaryotic parasites sharing metabolic pathways with human cells, including neoplastic cells. Hydroxyurea, a drug affecting ribonucleotide reductase enzyme, has been used to treat malignant and non-malignant diseases. Aims: 1) To determine if hydroxyurea inhibit *Leishmania* growth in vitro. 2) To develop a more accurate method that resembles the intracellular infection in the host. 3) To determine the ED_{50} of hydroxyurea on *Leishmania mexicana.* 4) To investigate the effect of hydroxyurea on the cell cycle of *Leishmania.*
Methods: Growth curve of M379 and Tab3 *Leishmania mexicana* strains were followed in the presence of 0.01, 0.1, 1, 10 and 100 μg/mL of hydroxyurea. To test the effect on intracellular parasites, adherent macrophages were infected with amastigotes-like forms, exposed to hydroxyurea by 3, 6, 9 or 12 days, when they were intracellular. Then, hydroxyurea was removed and parasites transformed to promastigotes by temperature shift from 32 to 26°C. Parasite density was monitored during 8 days. The ED_{50} was calculated by polynomial regression analysis. The cell cycle was studied in an EPICS-ALTRA flow cytometer.
Results: Hydroxyurea eliminated *Leishmania* at 10 and 100 μg/mL. The ED_{50} for intracellular parasites was 0.015 μg/mL, and for promastigotes was 0.05 μg/mL. Hydroxyurea at 10 and 100 μg/mL arrested *Leishmania* cell cycle at G_2/M.
Conclusions: Hydroxyurea is highly effective killing promastigotes and intracellular amastigotes in vitro. At the concentrations used, HU induces parasite death and cell cycle arrest of *Leishmania* in G_2/M.

Multiple Mechanisms of Action and Pharmacological Activities of Valproate

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Background: Although it is over 40 years since the anticonvulsant properties of valproate were discovered serendipitously, this drug remains one of the most widely prescribed anti-epileptics. Interestingly, several other indications have since emerged for valproate including mood stabilisation, prevention of migraine, treatment of mania and, most recently, chemotherapy of acute myeloid leukemias. In addition to these therapeutic effects, valproate is a known teratogen inducing neural tube defects in exposed offspring – an effect that is largely reflects its inhibition of cell proliferation. While the precise mechanisms of action that underlie these distinct pharmacological activities are unconfirmed, several different signalling pathways are influenced by valproate and related drugs.
Methods: The influence of valproate on cell proliferation, differentiation and cell cycle signaling was investigated in glioma (C6) and neuroblastoma (N2A and SHSY5Y) cell lines. Cell cycle synchrony was achieved by mitotic selection and western blotting techniques were employed to investigate the expression of cyclins and related proteins. The effect of valproate on cAMP signaling was investigated in forskolin-treated cells. These signaling pathways, along with other proposed mechanisms of action that may mediate the antiproliferative activity of valproate are reviewed and discussed.
Results: Valproate arrested cell cycle progression, induced ectopic expression of cyclin D3 and inhibited the accumulation of cyclic AMP via increased phosphodiesterase activity. Both these effects on cyclin and cAMP signaling involved perturbance in normal temporal G1 phase signaling mechanisms leading to cell cycle exit. Other mechanisms of action of valproate that may influence growth arrest include inhibition of histone deacetylase activity and activation of the tumour suppressor gene *PTEN via* peroxisome proliferator-activated receptors. The anticonvulsant activity of valproate may primarily reflect potentiation of GABAergic neurotransmission via multiple pathways.
Conclusions: Valproate is a clinically useful drug with a wide spectrum of pharmacological activity. Improved understanding of the mechanisms underlying its therapeutic and toxic effects will aid the development of new generation analogues with enhanced efficacy and reduced side-effects.

Photophysics, Photochemistry and induced-Photoallergy of Tricyclic Antidepressants

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Background: The major used tricyclic antidepressants (TCA) are the promazines, dibenzazepines, and dibenzodiazepines. Most of the derivatives of these drugs produce serious side effects, including *allergy and photosensitization*. Small changes in their structure, change their mode of action, potency and the spectrum and severity of the side effects. The molecular mechanisms for their photosensitizing ability are still unknown, even through these drugs are actually used in the world to treat thousands and thousands of psychiatric patients annually. The goals of this project are: 1) To measure the properties of their short-lived intermediates. 2) To find a molecular/photophysical descriptor for their phototoxic side effect.
Methods: The photophysical properties were measured in several solvents. In this work, we present absorption, steady-state, and time-resolved emission, laser flash photolysis, and quantum theoretical results for the ground state, the first excited singlet and triplet states, and the cation radical of several TCA series.
Results: The photophysical properties of the promazine family depend more on the solvent and the 2-substituents than on the dialkylaminopropyl chain. The largest effect was found for the triplet state of the 2-halogenated derivatives in phosphate buffer (PBS). The triplet state of these TCAs (^{3}TCA*) is efficiently quenched by a proton-transfer mechanism, and the rate of this quenching correlates very well with their phototoxicity. In the case of the imipramines, the ground-state properties are solvent-independent, while the emission maxima are red-shifted with increasing solvent polarity/polarizability. The fluorescence quantum yield is relatively low in all solvents.
Conclusions: 1) The effectiveness of the ^{3}TCA* quenching in PBS correlates very well with their phototoxicity index (i.e., the more effective the quenching, the smaller the triplet lifetime and the more phototoxic the drug). 2) Besides the ^{3}TCA*, the involvement of some membrane components is required to explain the large differences in phototoxicity of similar TCAs.

Anti-mycobacterial compounds: effects on the microbe and its host cell

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We study potentially anti-mycobacterial compounds in a cell culture system by looking at their effect on the intracellular survival of mycobacteria and innate immune response-related functions of the host cells.
We have established that lapachol, a naphthoquinone from *Tabebuia* sp., exhibits bacteriostatic activity toward *Mycobacterium avium* growing free as well as intracellularly in human THP-1 macrophage-like cells. Lapachol prevented free *M. avium* growth at a minimal inhibitory concentration of 32 mg/L (0.13 mM), which was also able to arrest intracellular bacterial growth while not being apoptotic toward the host cells. Regarding host cell function, we determined the effect of lapachol on the activation of THP-1 macrophages by IFN-□ and toll-like receptor 2 (TLR2) agonism. The induced expression of the NADPH oxidase catalytic component gp91-phox was decreased, while that of p47-phox and its translocation to the cell membrane were not affected. Some beneficial effects of lapachol on the host cells were observed: increases in IFN-□ receptor, ICAM and MHCII levels and a decrease in IL-10 secretion. IL-1□ production was not affected. The TLR2-mediated increase in TNF-□ secretion and in the levels of manganese superoxide dismutase (MnSOD), which protects the host cell from self-damage and apoptosis, was not impaired by the naphthoquinone. Instead, *M. avium*-induced TNF-□ secretion was decreased, probably as a reflection of the bacteriostatic effect of lapachol. We did not detect endoplasmic reticulum (ER) stress, as indicated by unchanged levels of grp78. Altogether, lapachol appears to be a satisfactory anti-mycobacterial agent.
The present report describes an in-vitro approach for the evaluation of new compounds with activity against intracellular microbes through the determination of relevant functional parameters of macrophages.

Antihormonals and liposomal carriers as novel strategies for chemo-radiotherapy based on cisplatin in cervical cancer.

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Background: Cisplatin (CP) is a widely used antineoplastic drug that has potent cytotoxic effects upon a variety of tumor types including cervical carcinoma. However, its administration is associated with nephrotoxic and neurotoxic events. On the other hand, steroid hormones are related to the development of drug resistance in cervical cancer. Upon this situation, in this work we have investigated the ability of a pure anti-estrogen ICI 182,780 (Fulvestran) and an anti-progestin Mifepristone (MF) to modulate the cytotoxic effect of CP and gamma irradiation in cervical cancer cell lines (HeLa and CaSki) and in a model of cervix cancer in athymic mice
Methods: The effect of CP alone and CP combined with either ICI, MF and/or gamma irradiation (RT) on cellular death was studied using an assay based on tetrazolium dye (XTT) and a clonogenic assay. Before and after treatment with antihormonals, expression of the estrogen, progesterone receptor (ER, PR) and the proangiogenic factor -vascular endothelial growth factor (VEGF)- genes were assessed by a reverse transcriptase polymerase chain reaction (RT-PCR). Cell-cycle modifications after combined treatments were studied by flow-cytometry. RT dose was evaluated with dosimetric procedures based on Gafchromic film.
Results*:* The analysis showed that ICI or MF alone produced no changes in cell growth; however, the combination of these antihormonals with CP and RT produced synergistic anti-proliferative effect in cervical cancer cells and significant delayed of the tumor growth without apparent toxic effect for the animals ($p<0.05$, $n=6$)). The effect of ICI and MF on the cytoxicity of CP and RT could be mediated, at least partially, by inhibition of ER, PR and VEGF gene expression, and by arresting the cell cycle at G2/M phase.
Conclusions: The results suggest that the combination of antihormonal drugs can improve the efficacy of CP and RT in cancer cells and tumor xenografts of cervical carcinoma. Based on these results we have planned the use of liposomes as drug carries of the CP and MF, which potentially could be used in chemoradiotherapy treatments decreasing the secondary effects of these drugs.

Combinatorial Ribosome-Inactivating Protein Libraries: Part of a New Arsenal in Combating Cancer

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Background: Ribosome-inactivating proteins (RIPs) such as ricin and Shiga-Like Toxin 1 (SLT-1) are highly effective at killing eukaryotic cells. Importantly, their cytotoxic A chain lacks receptor specificity. We propose that one can evolve a RIP A subunit template alone to specifically target and kill cancer cells by inserting a random peptide element within its structure and screening the resulting combinatorial library of A chain variants for RIP A chains displaying such selective killing properties. Aims: 1) To design a combinatorial SLT-1 A chain library expressing a repertoire of cytotoxic A subunit variants, each harboring a unique peptide ligand. 2) To purify recombinant SLT-1 A chain variants and identify single chain toxins able to kill human melanoma cell lines.
Methods: A combinatorial protein library was constructed by inserting a random heptapeptide element between residues 245 and 246 of the SLT-1 A chain. Single bacterial colonies were individually grown as 1mL cultures in 96-well blocks A His_8 affinity purification tag located at the N-terminus of all A chain variants was used to purify A subunit mutants from bacterial lysates using magnetic nickel NTA affinity beads. Aliquots of purified A chains were then dispensed into wells containing target cancer cells insensitive to wt SLT-1. Levels of cell survival were subsequently assessed using sulforhodamine B.
Results: 9,400 RIP A chain variants were individually purified and screened for their ability to kill human cancer cell lines insensitive to wt SLT-1 namely 518A2, PC-3 and CAMA-1 cells. 112 A chain variants were initially found to exhibit cytotoxicity towards one or more of these three cancer cell lines. The candidates were subsequently re-expressed and re-tested for cytotoxicity against a panel of normal and cancer cell lines. Only one variant was selectively toxic towards 518-A2 melanoma cells (CD_{50}, 300 nM) as well as towards 7 of 8 human melanoma cell-lines subsequently tested. This A chain was also effective *in vivo* in terms of causing tumor regression and animal survival when injected i.v.into SCID mice bearing a 518A2 xenograft.
Conclusions: The screening of combinatorial libraries of a ribosome-inactivating protein template represents a new strategy for identifying targeted protein-based anticancer agents that are distinct from antibodies.

A Climate for Change? A Statistical Analysis of General Practitioners' Relationship with Chiropractic Care.

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Introduction: Chiropractic is the complementary medicine dealing with correcting misaligned joints through mechanical manipulation. This study aims to identify the knowledge, attitudes and referral patterns of general practitioners (GPs) in Fife, Scotland regarding chiropractic care.
Materials and Methods: A postal questionnaire was sent to 100 GPs in Fife. Reply-paid envelopes were included, and a reminder questionnaire sent out one week later. GPs from ten randomly selected practices were asked to participate in a follow-up interview.
Results: Despite evidence demonstrating the usefulness of chiropractic, over 80% of GPs rated their knowledge of chiropractic as less than or equal to five out of ten (with 10 representing 'very knowledgeable'). The average score was three. GPs' attitudes towards chiropractic were fairly positive, though these attitudes were not reflected in GP perception of the helpfulness of chiropractic, or in their referral practices. In general, 3% of GPs found chiropractic very helpful, 14% rated it as helpful, 12% as neutral and 72% as unhelpful. GPs referred most readily for lower back pain, neck pain and sciatica. On average, 3% of GPs refer patients to chiropractors at the first consultation, 11% after failure of traditional treatments, 10% only at the patient's request, and 76% would never refer. Thirty-six percent of GPs would never refer patients to a chiropractor for any condition.
Conclusion: The results indicated an under-utilization of chiropractic treatment. GP attitudes towards chiropractic were positive; however, attitudes did not show a strong correlation with referral practices or with perceptions of helpfulness. GP perceptions of the conditions treated by chiropractic medicine do not match the evidence in this field. In general, GPs under-valued the helpfulness of chiropractic. However, for some conditions GPs perceived chiropractic to be more helpful than evidence-based medicine research supported. How helpful GPs perceived chiropractic to be was strongly correlated with the stage at which they were willing to refer patients to chiropractors. GPs seem very unwilling to refer patients to chiropractors; most will never refer or only refer at the patient's request. GP gender, years since qualification, number of patients per GP and number of GPs per practice did not significantly affect knowledge scores, overall attitudes towards chiropractic or mean number of monthly referrals to chiropractors.

Oxfendazole: A novel strategy to control Cystic Echinococcosis by targeting the intermediate host (sheep)

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Background: Cystic Echinococosis (CE) is a zoonotic disease caused by larval stage of the Echinococcus granulosus tapeworm. It is a major economic and public health problem worldwide. We determined the effects of high dose Oxfendazole, combination Oxfendazole/Praziquantel, and combination Albendazole/Praziquantel against CE in sheep.
Methods: A randomized placebo-controlled trial was carried out on 118 randomly selected ewes. Ewes were assigned to one of the following groups: 1) control; 2) OXF 60mg/Kg of body weight (BW) weekly for four weeks; 3) ALB 30mg/Kg BW + PZQ 40mg/Kg BW weekly for 6 weeks, and 4) OXF 30mg/Kg BW+ PZQ 40mg/Kg BW biweekly for 3 times (6 weeks). Percent protoscolex viability was performed using a 0.1% aqueous eosin vital stain for each cyst. "Cured" sheep were those that had no viable protoscolices; "improved" were those that had 1% to 60% protoscolex viability; and "unchanged" were those with more than 60% protoscolex viability. We evaluated 92 of the 118 sheep at the slaughterhouse.
Results: The CE prevalence was 95.7% (88/92) with a total number of cysts of 1094 (mean=12.4 cysts/animal). On average, the two-drug-combination groups had 6mm smaller pulmonary cysts than control ($p<0.05$) and 4.2mm smaller hepatic cysts than control ($p<0.05$). ALB/PZQ had the lowest PSC viability for lung cysts (12.7%), while OXF/PZQ had the greatest effect on liver cysts (13.5%). The percentage of either "cured" or "improved" sheep was 90%, 93.8% and 88.9% for OXF, ALB/PZQ and OXF/PZQ group as compared to 50% cured or improved for controls.
Conclusions: We demonstrate that Oxfendazole at 60mg, combination Oxfendazole/Praziquantel and combination Albendazole/Praziquantel are successful schemas that can be added to control measures in animals and could be used for the treatment of human CE. Further investigations on different schedules of monotherapy or combined chemotherapy are needed, as well as studies to evaluate the safety of Oxfendazole in humans.

Recombinant virus-like particles and their application for vaccine development and diagnostics

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Background: Supermolecular structures, named VLPs (virus-like particles), built symmetrically from hundreds of proteins of one or more types, represent molecules useful for the development of diagnostic, prophylactic and therapeutic tools for human and non human diseases. The aim of this study was to adapt the yeast expression system for polyomavirus and paramyxovirus nucleocapsid protein synthesis and VLPs production and to use those VLPs for diagnostics, monoclonal antibody generation, immunological investigations or virus basic research applications, as structural and assembly studies, receptor identification and entry studies.
Methods: A galactoseinducible *S. cerevisiae* yeast expression system was used. Formation of empty VLPs was confirmed by cesium chloride ultracentrifugation, agarose gel electrophoresis and electron microscopy. Recombinant VLPs were used for enzyme immunoassay studies, mice immunizations and monoclonal antibody generation using hybridoma technology.
Results: The high efficiency of the *S. cerevisiae*-based expression system was confirmed by the production of VLPs based on the VP1 of different human (JCPyV, BKPyV), primate (SV-40), mouse, hamster and avian polyomaviruses. The expression level of most polyomavirus VP1 proteins and mumps virus NP protein was high, and yielded 1-3 mg of purified protein per 1 g of wet biomass. Measles virus N protein yield was 6 mg/g of wet biomass and this showed the effectiveness of yeast as a host for generation of biomedical preparations. Measles and mumps virus nucleocapsid proteins were applied for commercial tests optimized for detection of these virus infections using oral fluids. Polyomavirus VP1-VLPs were used for diagnostics, structural and assembly studies and as molecular carriers of selected epitopes for production of chimeric VLP and the monoclonal antibody of desired specificity.
Conclusions: 1) We developed a universal expression system in yeast, for polyomavirus and paramyxovirus nucleocapsid protein synthesis and VLPs production. 2) The yeast generated recombinant viral VLPs retain biological activity of native virus proteins and were successfully used for detection and generation of specific antibodies.3). Polyomavirus VP1-VLPs were used for diagnostics, virus structure and entry studies and chimeric virus like particles production.

Development of Serpin drugs for the treatment of HIV/HCV co-transfections

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Background: Novel antivirals against HIV and HCV targeting host-cell proteins are needed to prevent the generation of multi-resistant viruses. The Serine protein inhibitors (Serpins) such as Secretory Leucocyte Protease Inhibitor (SLPI), anti-trypsin and Antithrombin III (ATIII), all display potent antiviral activity against HIV *in vitro*. Their *in vivo* potential can be demonstrated by: a) the near-absense of HIV oral transmission most likely due to the anti-viral activity of SLPI, the predominant HIV-inhibitor in saliva; b) the correlation between disease progression and certain anti-trypsin mutations; c) the observation that $CD8^+$ T cells of HIV long-term non-progressors produce a modified form of ATIII with high anti-viral activity. STUDY AIM: 1) Demonstrate anti-viral inhibition of ATIII for HIV and HCV; 2) Elucidate novel host-cell targets. ATIII is the first recombinant protein produced in goats and approved for human use. Due to its improved availablity, 60 h half-life and low toxicity ATIII has strong potential as a novel protein-based anti-viral against HIV and HCV.
Methods: HIV inhibition was measured in cell lines and human Peripheral Blood Mononuclear Cells (PBMC). HCV inhibition was measured using a replicon system with a full-length HCV genome. Activation or inhibition of pathways and host-cell targets was measured by microarray with 84 key genes testing for 18 different pathways.
Results: ATIII blocked HIV viral replication in nM and HCV in μM concentrations in a dose dependent manner. Using 2.4, 12 and 24 U/ml ATIII we saw 8 genes in HIV infected PBMC upregulated (Prostaglandin-endoperoxide (PTGS2), IL-8, IL-1α, CCL20, BCL2A1, MMP7, Fas and HK2). At the highest dose PTGS2 was 300-fold upregulated. IL-8 and IL-1α were both 60-fold upregulated. In the HCV replicon system seven genes were more than 10-fold downregulated. Cancer genes Jun and Myc where up to 1000-fold and 80-fold downregulated, respectively, transcription factor C/EBP was downregulated more than 600-fold.
Conclusions: ATIII blocks viral replication through a mechanism of action that targets multiple host-cell proteins which might decrease the ability of the viruses to develop resistance to this modality.

Development of Nonviral Gene Vectors for Gene Delivery to the Lungs and Blood Cells

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Background: Nonviral gene therapy could offer the opportunity to cure various inherited and acquired pulmonary and hematopoietic diseases. The aim of gene therapy is to: (1) design effective nonviral gene vectors, (2) optimize efficiency and specificity of transfer of genetic material into target cells, (3) minimize adverse toxicity and immune responses, (4) maximize the therapeutic potential.
Methods: Nonviral vectors, both episomal-replicating (S/MAR) and integration (ΦC31 integrase) based, were designed. Well-defined gene transfer agents possessing biodegradability, targeting functionalities and reduced toxicity were synthesized and applied via different routes into mice. Aerosol-based targeting of lung regions by combining gene vectors with magnetic gradient fields was investigated *in vivo*.
Results: Stable gene expression was achieved using different nonviral vectors together with plasmid DNA containing Ubiquitin C or Ubiquitin B promoters and *S/MAR* elements in hematopoietic cells *in vitro*. Stable expression in the lungs of mice was obtained with the co-delivery of ΦC31 integrase expression plasmid. When delivered as a fusion protein, recombinant ΦC31 integrase-TAT, mediated site-specific recombination in mammalian cells *in vitro***.** Using lactoferrin, insulin and clenbuterol as ligands coupled to PEI, selectivity towards specific cell types was achieved. Intranuclear trafficking of the plasmid into the nuclear matrix, resulted in higher and stable long-term expression in lung cells *in vitro*. High toxicity and non-biodegradability of PEI limit its *in vivo* application. Therefore, well-defined polymethacrylate based copolymers were characterized as gene transfer agents with low cytotoxicity, high colloidal stability and comparable transfection efficiency *in vitro*. Efficient methods for targeting of gene vectors to localized regions of the lung have been successfully established in mice by application of an external magnetic gradient field during inhalation of aerosols containing superparamagnetic iron oxide nanoparticles.
Conclusions: The standard requirements for clinical use of nonviral vectors have not been met yet in terms of efficiency and specificity. Future research will focus on improving the efficiency, specificity and safety of the gene delivery systems.

Pyrrole Hydrazones as a reliable Starting Platform in anti-tuberculosis Drugs Development

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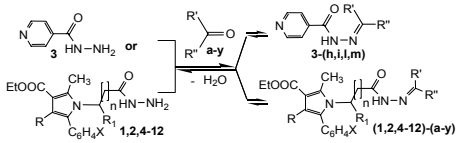
Background: The return of tuberculosis was declared by WHO as a global emergency. In the search for a synergic combination between the recognized anti-tubercular activity of both pyrrole derivatives and hydrazones, the current research offers sixty pyrrole-containing hydrazones synthesized and evaluated as potential tuberculostatics and 28 perspective new products designed thereof.
Methods: Both diversity and intermediate screening results directed the design of 88 hydrazones, prepared by condensation of Isoniazid or nine 1H-1-pyrrolylcarbohydrazides with 24 carbonyl compounds:



The products were evaluated at two levels High-throughput Screening (HTS): Level 1-screening against *M. tuberculosis* H37Rv (ATCC 27294) at 6.25 μg/mL in 12B medium using the Microplate Alamar Blue Assay and Level 2-determination of Minimum Inhibitory Concentration (MIC) of compounds with inhibition ≥ 90 %.
Results: 60 hydrazones exhibited inhibitory activity in Level 1 in the range of 0-100% and 12 hits were identified with 92-100% activity. Level 2 HTS pointed out 8 products with MICs=0.10-0.78, IC50=0.200-0.966, IC90=3.236-22.581 μg/mL and SIs=12.82-100 as candidates for further developments.
Some structure-activity correlations and a simplified second order QSAR-model were derived. Both R_1=CH_3 and electrono-withdrawing substituents in the aromatic ring decreased the activity. "Drug likeness" was assessed.
The architecture of some active hits was used as a template in the synthesis of new hydrazones of carbohydrazides **1, 2** and **4** and 28 perspective new analogs were prepared.
Conclusion: With inhibitory activities of 92-100% 12 compounds may serve as reliable prototypes in further ligand based design and optimization of potent anti-tubercular agents favoring compounds with moderate molecular sizes, polarity and hydrophobic parameters.

Authors' disclosure statement
The current research was performed in collaboration with the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF), Southern Research Institute, Frederick, USA, which organization performed the screening of compounds synthesized in our laboratory (acknowledged in the text). The results regarding the synthesis and evaluation of the disclosed 60 hydrazones were published recently in the following international journals:

- Bijev, A, Synthesis and preliminary screening of carbohydrazides and hydrazones of pyrrole as potential tuberculostatics, Arzneimittelforschung. 2006; 56(2): 96-103.
- Bijev A. New hydrazones as pyrrole derivatives with higher inhibitory activity against *Mycobacterium tuberculosis*. Lett Drug Des Discov. 2006;3(7):506-512.
- Bijev A. Synthesis, in vitro evaluations and structure-activity assessment of pyrrole hydrazones. Lett Drug Des Discov. 2008;5(1):15-24.
- Bijev A. Synthesis and in vitro Evaluation of New Hydrazones as Pyrrole Derivatives with Anti-tubercular Activity. Arzneim-Forsch/Drug Res. (2008, in press)

The essence of some latest unpublished results comprising structures and synthesis of 28 new perspective analogs whose design was based on the primary results completes the study.

Adoptive immunotherapy with Streptamer-selected HCMV specific T-cells after allogeneic stem cell transplantation

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Human cytomegalovirus (CMV) infection continues to be one of the most important and life threatening complications after allogeneic stem cell transplantation (SCT). Selective restoration of anti-CMV cellular immunity by CMV-specific adoptive T-cell transfer is an attractive alternative therapeutic approach, since it is an effective, non-toxic treatment. The finding that recovery of CD8+ CMV-specific cytotoxic T-cell (CTL) responses conferred protection against the development of CMV disease following allogeneic SCT stimulated attempts to restore antiviral immunity in humans. Different groups could demonstrate that chemotherapy-resistant CMV infections can be treated successfully with the adoptive transfer of *ex vivo* expanded CMV-specific T-cells. In principle, two different strategies to obtain sufficient amounts of T cells with defined specificity have been developed: *in vitro* expansion of T cell lines or clones, or the direct *ex vivo* purification of epitope-specific T-cells by multimeric HLA complexes. *In vitro* expansion is time consuming, and leads to a loss of antiviral activity and limited persistence of transferred immunity. Binding of multimeric complexes causes functional alterations of T-cells, and in addition, the cell preparation still contains the multimeric complexes used for isolation. Recently, the Streptamer technology was developed to overcome these problems. Streptamers are reversible multimers, which are unlikely to interfere with T- cell function, since Streptamer reagents can be rapidly dissociated from the T-cell receptor. Furthermore, T-cells selected by the Streptamer technology do not contain any reagents used for isolation. In this first clinical trial, we applied Streptamers to isolate functional CMV-specific CD8+ T-cells from stem cell donors. After complete dissociation of Streptamer reagents, the isolated T-cells are transferred directly - without any *in vitro* expansion - to the respective CMV-infected patients. First results of the ongoing phase I/II clinical trial show that sufficient numbers of specific T-cells could be selected from all CMV sero-positive stem cell donors. In addition, we initiated extensive clonal analyses to demonstrate that the transferred T-cells participate in cellular immune responses against CMV after transfer. In conclusion, transfer of specific T-cells selected by Streptamers is a safe and feasible method to restore CMV specific cellular immunity after allogeneic SCT.

Photodynamic therapy as a new method for the treatment of cutaneous leishmaniasis

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Background: Leishmaniasis is an important disease caused by Leishmania spp. Cutaneous leishmaniasis occurs within a few weeks after with a small papule on the exposed site and finally ulcerates. The drugs of choice, pentavalent antimony or meglumine antimonite are characteristically moderately toxic and there are risks of recurrence and unsatisfactory side effects. Leishmania was found deficient in at least five enzymes in the heme biosynthesis pathway. The first enzymes was delta-aminolevulinic acid (ALA). During the irradiation with red light the porphyrin-enriched tissues led to the cell death.
Methods: In this study, we used photodynamic therapy (PDT) for the treatment of cutaneous leishmaniasis caused by leishmania major..
In this study, the Leishmania lesions of five patients was applied locally with ALA 10%, then after 4 hours per treatment session was delivered, using red light (570–670 nm), 100 J/cm^2 at a light intensity of 150 mW/cm^2 (approximately 21 min). Treatments were repeated weekly for 4 times.
Results: In direct staining smears were showed no amastigotes after one or two sessions. The follow up continued for four months. The results showed that Photodynamic therapy to remodeling flattening and filling the sores.
Conclusion: Treatment of cutaneous leishmaniasis is directed toward the eradication of amastigotes and the reduction of the size of the lesions to promote healing and achieve maximum efficacy with minimal scarring and toxicity. Photodynamic therapy in contrast to all systemic treatment modalities has no risk of toxicity but only mild local inflammatory reaction with an excellent cosmetic outcome. So PDT might offer a new promising treatment modality for the disseminated lesions of cutaneous leishmaniasis.

Designing Material of Particular Equilibrium and Transport Properties by *Ab Initio* Molecular Dynamics

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Background: Prediction and study of equilibrium and transport properties of ionic liquids as green solvent are currently of high interest. These liquids are used in organic synthesis without harmful environmental effect of ordinary volatile solvent. Application of atomistic simulation methods to design 1-alkyl-3-methyl imidazolium based ionic liquids of suitable equilibrium and transport properties as well as understanding the aggregation mechanism within the ionic liquid assembly forms the aims of the present study.

Methods: *ab initio* Car-Parrinello molecular dynamic (CPMD) simulation was used to simulate the transport properties including viscosity and diffusion constant of 1-alkyl-3-methyl imidazolium based ionic liquids. Simple anion like chloride (Cl^-) and iodide (I^-) and complex ones like BF_4^- and PF_6^- was studied. All the simulations were made at 300K. In the same way the aggregation of the ionic liquids with long chain alkyl group was studied. A thorough understanding of the dynamic and the structure were followed by studying the static properties by using Gaussian program.

Results: Structural and the dynamic properties were studied by the results of simulation based on statistical mechanics of the liquid state. The studies show a slow dynamic, higher viscosity, smaller coordination number for chloride compounds than the iodide one. The I^- compound form larger aggregates than the Cl^- one. Contrary to the ionic liquids with Cl^- and I^- anions, the BF_4^- and PF_6^- compounds show hydrophobic properties, and therefore they show higher dynamics, smaller viscosity, and those properties characteristics of tightly bonded ion pair salts.

Conclusions: The slow dynamics and high viscosity of the imidazolium based ionic liquids containing simple anions are due to the strong electrostatic cation-anion interaction. The more complex anions make a more complex interaction leading to the hydrophobic property. The details knowledge of these properties enables to design system of interest with desire equilibrium and transport characters.

Buccal Delivery of Carbamazepine (CBZ): a New Scenario in Management of Trigeminal Neuralgia (TN)

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Background: CBZ is one of the most effective drugs in treatment of TN; however, on chronic dosing, the drug presents a decrease in its half-life due to metabolism autoinduction. Following *peroral* administration, bioavailability of CBZ is limited by poor water solubility. Moreover, in medications, the various coexisting polymorphs of CBZ generate great fluctuations in drug solubility and absorption. The entrapment of CBZ into polymeric microspheres (MS) might alter the crystal habit of CBZ and provide regular dissolution and improved bioavailability.
The oral cavity is an attractive site for drug delivery due to ease of administration, and avoidance of first-pass metabolism and possible drug degradation in gastro-intestinal tract. Buccal tablets prepared with CBZ loaded MS were developed to obtain slow and regular drug release.
Methods: The aptitude of CBZ to penetrate porcine buccal mucosa and reconstituted human oral epithelium (HOE) was evaluated using Franz diffusion cells and Transwell diffusion cells system respectively (donor phase drug solutions in artificial saliva, acceptor phase artificial plasma). CBZ loaded MS were prepared by the emulsion solvent evaporation method using Eudragit® L-100 as polymer. CBZ-polymer interactions were measured by differential scanning calorimetric analysis. Tablets for transbuccal CBZ administration were prepared by direct compression of drug loaded MS. CBZ release from tablets was performed *in vitro* using a flow through system in conditions simulating the oral cavity environment.
Results: CBZ well penetrates the buccal membrane: drug fluxes and permeability coefficients were calculated as $7 \cdot 10^{-2}$ mg/cm^{2}h and 0.23 cm/h for HOE and $1.81 \cdot 10^{-2}$ mg/cm^{2}h and $4.57 \cdot 10^{-2}$ cm/h for porcine mucosa respectively, thus suggesting that buccal mucosa does not appear a limiting step to the drug absorption. The thermogram of CBZ loaded MS confirmed the complete drug amorphization that implies regular drug solubilization. The release of CBZ from tablets showed a reproducible Higuchian pattern.
Conclusions: Buccal mucosa does not block diffusion of CBZ and could represent an alternative way of the drug administration. CBZ can be successfully transformed into loaded Eudragit® L-100 MS that, in turn, after direct compression, can form tablets useful for slow pre-programmed drug delivery on buccal mucosa.

Treatment of Intravenous Drug Users with Chronic Hepatitis C: Treatment Response, Compliance and Side Effects

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Background:Although IVDUs comprise the majority of patients with chronic hepatitis C, most of them are excluded from treatment because of concerns about adherence to treatment and side effects.
Methods: In this study we retrospectively evaluated safety, compliance to treatment and efficacy of treatment in IVDUs with HCV infection in 163 former IVDUs with chronic hepatitis C, who were not in methadone substitution and were attending our clinics during 1997-2004. All subjects were HCVRNA (+), had ALT levels >X1,5 UNL and were treated for their HCV infection. Treatment consisted of three different regimens: IFN-α monotherapy (39,8%), IFN-α/ribavirin combination therapy (30,1%) and pegylated IFN-α/ribavirin combination therapy. 87/163 patients (53,3%) discontinued treatment early due to drug abuse relapse (62%), side effects (32,1%, 10% psychiatric) and 5,7% for other reasons. 80% of those who discontinued treatment had pre-treatment drug abstinence ≤ 9 months. 70/76 patients who completed therapy had an end-of-treatment virologic response (ETR, 92%). 54/76 patients showed sustained virologic response (SVR, 71,05%).
Results: ETR and SVR were significantly higher in both combination therapies compared to IFN-α monotherapy. The most prevalent HCV genotype was 3 (65%) and mild histological lesions were detected in the majority of subjects. In conclusion our findings show that treatment for chronic hepatitis C was reasonably safe and sufficiently effective in our group of non methadone-substituted IVDUs, despite the fact that more than half of them discontinued treatment early and many relapsed to drug abuse. We suggest that the optimal duration of pretreatment abstinence from drug abuse should be $\geq$ 9 months.

Evidence for Anti-Cancer activity for the Antidepressant Sertraline, *In-Vitro* and *In-Vivo* Effect in Nude Mice Xenografted with HT29 cells.

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Background: Recent reports provide evidence for a pro-apoptotic activity of some antidepressants, mainly the serotonin reuptake inhibitors (SSRIs). Oncogenes like Bcl2, and proteins of the mitogen activated protein kinases (MAPK) pathway, participate in the pathogenesis of the disease. Objectives: 1. Evaluation of the effect of SSRIs on apoptosis markers 2. Determination of the molecular mechanism of the drugs. 3. Evaluation of the *in vivo* effect of the SSRIs in a nude mouse model xenografted s.c with human colorectal carcinoma cells HT29.
Methods: Human colorectal carcinoma HT29, and LS1034, a multi drug resistant (MDR) cell lines (ATCC) were studied. Cell viability (neutral red) and cell proliferation (^{3}H-thymidine incorporation) were determined. Apoptosis was studied using flow cytometry of propidium iodide stained cells and caspase 3 determination by an enzymatic fluorimetric assay. Protein expression was determined by western blot analysis. Tumor growth was determined in CD1 nude mice xenografted s.c and treated ip with the drugs.
Results: The SSRIs paroxetine and sertraline induced a dose-dependent inhibition of cell viability and proliferation in both cell-lines (IC50 8-15mcM). When compared to some cytotoxic agents e.g doxorubicin, vincristine and 5FU, the SSRI's activity demonstrated a similar (HT29) or stronger effect (LS1034). Both agents stimulated DNA fragmentation and increased caspase-3 activation, suggesting a proapoptotic mechanism. Western blot analysis revealed an increase 24hr later in c-Jun and p-ERK and decreased Bcl2 expression. For in vivo experiments, we used CD1 nude mice xenografted subcutaneously with HT29 cells. Sertraline (3 times/week 15mg /kg s.c or i.p), but not paroxetine, induced a significant inhibition of tumor growth the animals.
Conclusions: Collectively, our results suggest that the widely used and safe antidepressant sertraline possesses potential anti-tumor activity, which circumvents the MDR mechanism and thus could be valuable in the arsenal of colon carcinoma therapy. Since SSRI therapy is frequently indicated in cancer patients as an antidepressant, this possibility seems attractive.

Serotonin Transporter: Mechanisms of Inhibition by Enteropathogenic *E. coli* (EPEC)

GILL R, ESMAILI A, NAZIR S, BORTHAKUR A, TURNER J, ALREFAI W, HECHT G, DUDEJA P.

Background: Serotonin transporter (SERT) plays a critical role in regulating serotonin availability by its reuptake through a Na^+ and Cl^- coupled mechanism. Elevated levels of serotonin are associated with several diarrheal conditions including inflammatory bowel diseases and enteric infections. However, whether alteration in SERT activity contributes to the pathophysiology of diarrhea induced by food-borne pathogens such as enteropathogenic *E coli* (EPEC) is not known. EPEC is non-toxigenic but houses a pathogenicity island encoding a type III secretory system (T3SS) that translocates bacterial proteins directly into host cells. We hypothesized that EPEC decreases SERT activity to contribute to the associated rapid diarrhea. Therefore, present studies were aimed at examining the effects of EPEC infection on SERT activity and expression and delineating the underlying mechanisms.
Methods: Caco-2 cells were used as an *in vitro* model of human intestinal epithelia and were infected with EPEC strain E2348/69 or commensal *E. coli*. SERT activity was measured as fluoxetine-sensitive ^{3}[H]-5-HT uptake. Infection of Caco-2 cells with EPEC for 30-90 min decreased luminal SERT activity (~50-60% inhibition at 30 min; P<0.005); however, infection with commensal *E. coli* had no impact. Kinetic analysis revealed that EPEC infection inhibited SERT activity via a decrease in V_{max} (~ 3 fold). In parallel, EPEC infection caused internalization of SERT from the plasma membrane to endocytic vesicles as assessed by live cell imaging of SERT-GFP construct in transfected Caco-2 cells. Mutation of *escN*, which encodes the ATPase for T3SS, ablated the effect of EPEC on luminal SERT activity indicating that effects of EPEC were T3SS dependent. Inhibitory effect of EPEC on SERT activity was abolished in the presence of tyrosine phosphatase inhibitors (phenyl arsine oxide and dephostatin). EPEC infection in vivo significantly reduced mucosal 5-HT content in the mouse small intestine.
Conclusion: Infection of intestinal epithelial cells with EPEC decreases SERT via a T3SS dependent mechanism and involvement of tyrosine phosphatases. These data further highlight the interactions of a common enteric pathogen with the expression and function of SERT and provide mechanistic insights into development of a potential new pharmacotherapy to modulate the serotoninergic system in treatment of diarrheal diseases.

A new mode of cell death for tumor cells after ascorbate : menadione treatment in vitro and in vivo.

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Background: Dietary supplements can be used as adjuvants to treat several kinds of cancers. Nucleases are repressed in tumors. Reactivating nucleases in tumor cells would assist in killing those cells. Tumor-bearing rodents fed by a mixture of ascorbate (VC) + menadione (VK3) before irradiation or chemotherapy brings a higher rate of survival and significant decrease of tumor size.
Methods: Series of 10^6 human carcinoma cells (DU145, T24, RT4, MDAH, etc) grown on 12 mm diam. titer dishes with 24 h in M5A milieu, washed with PBS saline, overlaid for 1, 2, 4, or 6h with 2 mL M5A with VC, VK3 or VC+VK3 + 2 mL PBS (cytotoxic doses CD_{99}) washed by PBS, then prepared for electron microscopy. Male 4 mths old, NCr-*nu/nu* mice injected with 10^6 DU145 cells in 100 µL M5A medium were injected sc. After 4 wks, given 100 µL PBS or 100 mL VC+VK3 and killed 1, 2, 4, 8 and 24h after injection, and 1-4 mm tumors on diaphragm were prepared for TEM.
Results: Carcinoma cell treated by Sham-PBS, VC, VK3, VC+VK3 combinations for 1, 2 and 4h show the cytotoxic damages by electron microscopy, biochemistry and flow cytometry were treatment-dependent as VC+VK3 > VC > VK3 > Sham. Oxidative stress induces alterations of cytoskeleton, mitochondria, lysosomes lead to cytoplasm self-excisions without organelles. Cell size reduction and other nuclear damages, DNAses I + II reactivation, with DNA gel electrophoreses smears patterns are data consistent with injuries of autoschizic cell death, not apoptosis. In vivo DU145 tumors treated reactivated DNAses and morphology reveals tumor demise by autoschizis causing tumor shrinkage and significant mice survival.
Conclusions: VC: VK3 (or VC) exerts antitumor activities through a wide array of mechanisms: oxidative stress, nucleases' reactivation, cell cycle blocks and induction of autoschizic cell death (Gilloteaux et al., 1995-2006). Metalloproteinases inhibition and potentiation of the immune system make VC+VK3 combination, or Apatone ® , a magic bullet to be used safely in patients against many tumor cells.

Erythropoietic Stimulating Proteins - What is the Optimal, Safe Hemoglobin Target?

GILMARTIN C

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Background: Erythropoietic stimulating proteins (ESPs) have been used in the treatment of anemia of chronic kidney disease (CKD) and chemotherapy-induced anemia (CIA) since 1989 and the early the 1990s, respectively to a target hemoglobin (HgB) between 11 to 12 g/dL. Recent clinical trials have examined HgB targets > 12 g/dL that have demonstrated increased mortality and thrombotic events in both anemia of CKD and CIA. ESPs have also been utilized for the off-label indication in anemia of cancer.
Methods: A review of the clinical trials prompting the addition of the boxed warning to the ESPs product labeling in the United States was performed. An evaluation of the trials including target Hgb, primary endpoints and outcomes in both CKD, CIA and anemia of cancer was assimilated.
Results: The following table summarizes the trials in CKD anemia.

Reference (Study)	Hgb (g/dL) Target	Primary End Point	Outcome	P value
Singh et al 2006 (CHOIR) N = 1,432 CKD not on dialysis	13.5	Composite of death, myocardial infarction (MI), or congestive heart failure without dialysis or stroke	Composite events were higher in ESP treated group	0.03
Drüeke et al 2006 (CREATE 1998) N = 603 CKD not on dialysis	13 - 15	Time to first cardiovascular (CV) event	No benefit in ESPs to first CV event	0.20
Besarab et al 1998 (NI HCT) N = 1,233 CKDon dialysis with cardiac disease	Hematocrit 42% ± 3%	Time to death or first nonfatal MI	Trend to higher rate of death or MI in patients treated to higher hematocrit	0.48

The table below summarizes the trials for CIA and anemia of cancer
[Regrettably not enough space on this page.]
Conclusions: Targeting Hgb levels ≥ 12 g/dL CKD and CIA utilizing ESPs has not shown benefit and may increase the risk of death and thrombotic events. CIA trials demonstrated a decreased progression free and overall survival and decreased locoregional tumor control with ESP use. One trial failed to appreciate a reduction in RBC transfusions with ESP therapy. ESPs for CIA are indicated only concurrently with mylosuppresive therapy when cure is not the goal. Current guidelines recommend initiation when the Hgb < 10g/dL. Guidelines in CKD recommend initiation when < 11 g/dL, and caution when approaching 13 g/dL.

High-dose cyclophosphamide is active in immune-mediated illnesses.

GLADSTONE D

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Background: High-dose cyclophosphamide is active in immune-mediated illnesses.
Objective: To describe the effects of high-dose cyclophosphamide on severe refractory multiple sclerosis.
Design, Setting, and Patients: Patients with multiple sclerosis with an Expanded Disability Status Scale (EDSS) score of 3.5 or higher after 2 or more Food and Drug Administration-approved disease-modifying therapy regimens were evaluated.
Intervention: Patients received 200 mg/kg of cyclophosphamide over 4 days.
Main Outcome Measures: Patients had brain magnetic resonance imaging ad neuro-ophthalmologic evaluations every 6 months and quarterly EDSS and quality-of-life evaluations for 2 years.
Results: Twelve patients were evaluated for clinical response (median follow-up,15.0 months; follow-up range, -24 months). During follow-up, no patients increased their baseline EDSS scores by more than 1.0. Five patients decreased their EDSS scores by 1.0 or more (EDSS score decrease range, 1.0-5.0). Two of 11 patients had a single enhancing lesion at baseline; these lesions resolved after high-dose cyclophosphamide treatment. At 12 months, 1 patient showed 1 new enhancing lesion without a corresponding high-intensity T2-weighted or fluid-attenuated inversion recovery signal. Patients reported improvement in all of the quality-of-life parameters measured. Neurologic improvement involved changes in gait, bladder control, and visual function. Treatment response was seen regardless of the baseline presence or absence of contrast lesion activity. Patient quality-of-life improvement occurred independently of EDSS score changes. In this small group of patients with treatment-refractory multiple sclerosis, high-dose cyclophosphamide was associated with minimal morbidity and improved clinical outcomes.
Conclusions: High-dose cyclophosphamide treatment in patients with severe refractory multiple sclerosis can result in disease stabilization, improved functionality, and improved quality of life. Further studies are necessary to determine the most appropriate patients for this treatment.

Vancomycin Resistant Enterococcus faecium Under Constant Linezolid Exposure

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Background: The occurence of vancomycin resistant Enterococcus (VRE) has been increasing from 2.7% to 13.5% in Germany (2002-2004). This is due to too high, too low and inadequate dosing of antibiotics.[1] For VRE colonised patients linezolid offers an alternative treatment. The prediction of the antibacterial effect by a pharmacokinetic/pharmacodynamic (PK/PD) analysis could guide the antibiotic therapy, shorten the duration of disease and reduce the extent of bacterial resistance.
Methods: The killing behaviour of VRE (ATCC 700221) over time under various linezolid concentrations was investigated in a static *in vitro* model. An inoculum of 106 cfu/mL VRE in Mueller-Hinton (MH) broth was spiked with linezolid concentrations of 0.5, 1, 2, 4, 8, 16, 32 µg/mL, incubated at 37 °C and continuously shaken (62 min^{-1}). The time-killing process was monitored via viable cell counting over 24 h. Bacteria samples were taken at 0, 1, 2, 4, 6, 8, 10, 12, 16, 20 and 24 h and plated on MH agar plates with 5% sheep blood. After 24 h of incubation all plates were counted by a digital automatic colony counter (ColonyQuant, Schuett Biotec, Goettingen, Germany). Geometric means and confidence intervals of the respective bacterial concentrations were numerically calculated via bootstrapping in Excel (Mircosoft).
Results: The time-kill curves of VRE under various linezolid concentrations were investigated. Linezolid concentrations of 4 and 8 µg/mL were identified as bacteriostatic and 17 and 36 µg/mL as bactericidal. For concentrations lower than 4 µg/mL bacterial regrowth after 1h was visible. Moreover, 17 and 36 µg/mL achieved very simlar rates in bacterial killing.
Conclusions: Longer-term in vitro time-kill curves for VRE under various linezolid concentrations describe the bacterial growth. LZD displayes time- and concentration-dependent effects of killing with respect to VRE.

1. Kresken, M., et al., PEG-Resistenzstudie 2004. 2006, Paul-Ehrlich-Gesellschaft für Chemotherapie e.V.: Rheinbach. p. 1-90.

Antibody responses in cancer vaccines and immunotherapies: from cancer/testis antigens to new targets discovered by protein arrays

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Background: Analysis of antibody responses to self-antigens has driven the development of the field of tumor immunology. While cancer/testis antigens have been put to the test in clinical trials, more targets are needed. Protein microarray technologies offer an unprecedented platform to assay the serological response of cancer patients to tumor antigens in a comprehensive fashion.
Methods: Sera from patients with non-small cell lung cancer (NSCLC), epithelial ovarian cancer (EOC), and pancreatic cancer, as well as healthy donor sera from the New York Blood Bank, were collected and tested by Enzyme-Linked ImmunoSorbent Assay (ELISA) against known recombinant tumor antigens, as well as by antibody profiling using commercially available protein microarrays containing >8200 antigens.
Results: We first validated our approach by using sera with known immunoreactivity in ELISA to antigens present on microarrays. We found 197 antigens reacting frequently and strongly with EOC patient sera compared to healthy donor sera. The same study with pancreatic cancer patient sera returned 28 antigens with preferred immunogenicity in cancer, 21% of which overlapped with antigens immunogenic in EOC.
Conclusions: With a stringent strategy for data analysis and normalization designed to determine antigen-specific serum antibody responses using protein arrays, we describe new antigens immunogenic in cancer patients and propose that this approach is suitable for defining potential antigenic targets for cancer vaccine development, serum antibody signatures with clinical value, characterization of predictive serum markers for experimental therapeutics, and eventually for the serological definition of the cancer proteome (seromics).

Intravascular fluid replacement in the critically ill: Is it the substance or the timing that makes the difference?

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Background: The ideal infusion solution used for intravascular fluid replacement in critically ill patients is still not determined in order to improve patients´outcome. Further, inconsistent opinions exist whether restrictive or liberal fluid management is beneficial in critical ill patients.
In this regard, also the "timing aspect", and how to guide fluid therapy in critically ill patients has not been elucidated yet satisfactorily. We therefore performed a prospective study in cardiac surgery patients where the timing and the amount of fluid replacement was guided by an early goal directed hemodynamic algorithm using volumetric parameters of cardiac preload for optimization of timing and quantity of intravascular fluid replacement.
Methods: 40 patients undergoing cardiac bypass surgery were included prospectively (study group, SG) and compared with a control group (CG). In the SG, from induction of anaesthesia until 48 hours after surgery, hemodynamic management was continuously guided by an algorithm based on the measurement of global enddiastolic volume index (GEDVI) and cardiac index (CI). Hemodynamic goals were a GEDVI > 640 ml m^{-2}, CI > 2.5 l m^{-2}, and mean arterial pressure (MAP) > 70 mmHg. The CG was treated at the discretion of the attending physician based on central venous pressure (CVP), MAP and subjective clinical evaluation.
Results: Total duration of catecholamine and vasopressor dependency was significantly shorter in the SG (187 ± 70 min vs. 1458 ± 197 min, $p < 0.001$). Less vasopressors (0.73 ± 0.32 mg vs. 6.67 ± 1.21 mg, $p < 0.001$) as well as catecholamines (0.01 mg ± 0.01 mg vs. 0.83 ± 0.27 mg, $p < 0.001$) were administered in the SG. In cristalloid infusions no differences were detected at any time. In the SG significantly more colloids (HES 130/0.4, gelatin solution 3.5%) were used during surgery and ICU-therapy (1515 ± 60 ml of colloids vs. 1327 ± 50 ml during surgery and 5403 ± 222 ml vs. 4187 ± 167 ml during ICU therapy). Regarding allover fluid bilance (6509 ± 240 ml SG vs. 6403 ± 184 ml CG; $p < 0.05$) there was no clinical relevant difference. SG patients reached ICU-discharge criteria significantly earlier (25 ± 13 vs. 33 ± 17 h).
Conclusions: Not only the substance used for fluid replacement but continuous preload optimization and the optimal timing for fluid application are necessary to improve critical ill patients outcome.

Changing the dosing frequency of ceftazidime transforms this "Daisy cutter" into an antibiotic with limited collateral damage while retaining its efficacy

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Background: During treatment of infection the host's normal flora is unintentionally exposed to antibiotics, which may lead to secondary colonization by potentially pathogenic, often multiple antibiotic-resistant, organisms. The aims of the present investigation is to study in rats the effect of ceftazidime dosing increments and frequency of dosing on the selection of ceftazidime-resistant *Enterobacter cloacae* in the intestine during treatment of a pulmonary infection caused by *Klebsiella pneumoniae.*
Methods: Rats with pulmonary infection (n=10 per group) received therapy with doses of 3.1 to 400 mg/kg/day of ceftazidime at a frequency of every 6,12 or 24 h during 18 days, starting 24 h after bacterial inoculation of the lung. Emergence of resistance in intestinal *E. cloacae* was monitored by culturing fresh stool specimens at days 0, 8, 15, 22, 29, 36 and 43 on agar plates with (6.4 mg/L) and without ceftazidime. Pharmacodynamic indices and time within the mutation selection window (MSW) were assessed in infected rats for each regimen. Ceftazidime-resistant *E. cloacae* mutants were characterized by determination of the ß-lactamase activity under cefoxitin-induced and non-induced conditions.
Results: A reduction of intestinal ceftazidime-susceptible *E. cloacae* was observed and showed a significant correlation with the *f*AUC/MIC at days 8, 15 and 22 and with the fC_{max} on days 8, 15, 22, 29 and 36, respectively. More rats treated with 12-25 and 50-100 mg/kg/day every 6 h were found colonised with ceftazidime-resistant *E. cloacae* mutants than animals treated every 12 h or every 24 h. The proportion of rats colonised with ceftazidime-resistant *E. cloacae* mutants at days 15, 36 and 43 correlated with the time during which ceftazidime-plasma concentrations were within the boundaries of the MSW. Ceftazidime-resistant *E. cloacae* mutants (MIC $\geq$ 128 mg/L) were characterized as stable derepressed mutants. Furthermore, the therapeutic efficacy on the *K. pneumonia* pulmonary infection by long ceftazidime treatment appeared to correlate with the *f*AUC/MIC ratio (AUC is the area under the concentration time curve).
Conclusions: Colonization with stable derepressed ceftazidime-resistant *E. cloacae* mutants particularly occurred when rats were exposed to moderate doses of ceftazidime (12-25 or 50-100 mg/kg/day) administered every 6 h. Emergence of resistance was correlated to time within the MSW.

Frovatriptan - the triptan with the least drug/drug interactions

GOLDSTEIN J

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Frovatriptan was the sixth triptan to be approved since the approval of Sumatriptan (Imitrex/Imigran) in 1993. Concern over cardio-toxicity and drug/drug interactions has prompted development of other triptans. Also the short duration of action of most triptans (2-4 hours) has encouraged the development of other triptans. In addition to the cerebro-selective nature of Frovatriptan multiple clinical trials have confirmed tolerability and efficacy with statistically significant results of efficacy. Frovatriptan has no inhibiting or inducing effects on cytochrome P450 enzymes and therefore has the lowest potential for drug/drug interactions of all the triptans. Clinical trials confirm that Frovatriptan can be administered safely in patients with hepatic and renal impairment and there is no specific age limit to administration of the drug. Low recurrence rate has also been confirmed in clinical trials and Frovatriptan has been studied in menstrual migraine headache with demonstrable efficacy in that condition. Thus, Frovatriptan is the triptan best suited for long duration headaches, headaches prone to recurrence, and those patients with significant triptan side effects.

Molecular Imaging of Multidrug Resistance by ABC Transporters in Osteosarcoma

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Background: Multidrug resistance (MDR) is a significant obstacle to successful chemotherapy and a major prognostic factor in osteosarcoma (OS) patients. We have previously demonstrated that the sensitivity of OS cell lines to doxorubicin and cisplatin depends on MDR-related ABC-transporters and can be predicted based on functional assays using ^{99m}Tc-Sestamibi (MIBI). These observations prompted us to develop an orthotopic model of OS to evaluate the role of MIBI imaging in the assessment of MDR and pharmacological inhibition.
Methods: Sensitive (143B) and resistant (MNNG) OS cell lines expressing different levels of P-glycoprotein carrying a luciferase reporter gene were inoculated into the tibia of nude mice. Local tumour growth was monitored weekly by whole-body bioluminescent reporter imaging and by radiography. After primary tumour growth, the animals were imaged with MIBI during 60 min. A group of animals were pre-treated with a Pgp inhibitor (PSC833). Images were analyzed for calculation of MIBI washout half-life ($t_{1/2}$), % of washout rate (%WR) and uptake ratio.
Results: A faster washout rate of [^{99m}Tc]MIBI was observed in resistant tumours has demonstrated by the shorter $t_{1/2}$ and higher %WR in MNNG-resistant tumors ($t_{1/2}$=87.3±15.7min; %WR=37.5±4.0%) compared with those in 143B-sensitive tumors ($t_{1/2}$=161.0±47.4min; %WR=24.6±7.5%). Administration of PSC833 increased significantly the retention of MIBI in MNNG tumors ($t_{1/2}$=173.0±24.5min, %WR=23.8±4.3%, $p<0.05$) and had no significant effects on 143B-sensitive tumours.
Conclusions: 1) The orthotopic injection of cancer cells provides an animal model closely resembling the clinical situation of OS that can be used for functional imaging of MDR. 2) The kinetic analysis of MIBI washout provides information on the functional activity of MDR-related to Ppg expression and its pharmacological inhibition. 3) Functional imaging with MIBI might be a valuable clinical tool to predict chemotherapy response in OS.

Antioxidant Supplementation Impacts upon Electronegative Low Density Lipoprotein Plasma Levels and Cardiovascular Risk

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Oxidative stress has been considered determinant of aging and other pathological processes. Indeed a number of authors have shown a decrease in non-enzymatic antioxidants during aging. Moreover, human chronic diseases, as cardiovascular and neurodegenrative diseases are also proposed to have an oxidative stress component. Despite various attempts to prove the above statement, researchers have been poorly successful. The failure to demonstrate the role of antioxidant strategies against the mentioned diseases has many causes, mainly the lack of the antioxidant network knowledge to design an appropriate strategy. The present paper deals with decreasing levels of minimally oxidized LDL in urban hypercholesterolemic elderly patients. Antioxidant supplementation to hypercholesterolemic patients, had the following composition: Tablet 1:600 mg Vitamin C, 200 mg Vitamin E, 0.6 mg β-carotene; 40 mg Zinc; 1.0 mg Copper; and 100 μg Selenium; Tablet 2: 10 mg Coenzyme Q10. Participants were divided into five groups (20 per group) and received a combination of the two tablets or placebo for 180 days. It was demonstrated that plasma levels of antioxidant vitamins E, C and β-carotene were increased among the subjects taking two tablets 1. Q10 coenzyme was increased only among those taking two tablets 1 and one tablet 2. Minimally oxidized LDL levels decrease was dependent on the intake of two tablets 1. Lower intakes were ineffective. In conclusion, an adequate antioxidant strategy decreases oxidative stress. Whether it may offer a potential protection against vascular diseases, it remains to be studied in large controlled trials. Financial support: Fapesp, CNPq, CAPES, and Marjan Indústria Farmacêutica.

NO-Donors As Modulators of Antitumor Drugs Resistance.

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One of the major causes of anticancer chemotherapy failure is connected with development of resistance of tumors to those drugs to which they were high sensitive at first. According to famous **P.Ehrlich**, "tumor resistance follows chemotherapy like a shadow, and brings to naught all its achievements and successes". And it is indeed so. The resistance is developing to all known drugs, including the latest targeted ones. Based on modern knowledge of drug resistance (DR) mechanisms, many compounds were proposed to overcome it. But, almost all of them did not found the use because of high toxicity. And the quest for DR modulators is continued.
Last time an important role of nitric oxide (NO) in different biological processes, including tumor growth, was elucidated. NO participates in the effect of antitumor drugs. Some works link NO and DR. These data stimulated the use of exogenous NO-donors in chemotherapeutic studies, including those concerning DR.
We investigated the possibility of using two NO-donating compounds: 1)AK-2123 {N-(2'-me-thoxyethyl)-2-[3"-nitro-1"-triazolyl]acetamide}, has 1 NO-group; 2) NMO [3,3-bis(nitroxymethyl)ok-setan}, has 2 NO-groups.
We showed that AK-2123 significantly enhanced the sensitivity of multidrug-resistant (MDR) strains of P388 mouse leukemia (developed and characterized on phenotype and genotype by us) to mitomycin C (MMC). The modulating effect was dependent on the initial sensitivity of resistant tumors to MMC, which was correlated with existence or absence in MDR-amplicon of sorcin gene coamplification. Moreover, AK-2123, used in extra low doses (10^{-6}–10^{-10} mg/kg) also increased the sensitivity MDR-tumors to MMC.
NMO essentially slows down of resistance forming of parent P388 tumor to cyclophosphamide (CPA), from 8th to 14th transplant generation.
On different experimental models we discovered that both compounds increased the antitumor and antimetastatic effect of various traditional anticancer drugs (CPA, doxorubicin, cisplatin), used in low uneffective doses.
Thus, the combined employ of NO-donors and cytostatics may slow the development of DR to the latter and help in cure of MDR-tumors. Beside, NO-donors may be used as adjuvant agent in the chemotherapy of tumors.
In our opinion, our data show that NO-donating compounds could be excellent supplement to **P. Ehrlich's MAGIC BULLET**, when it is found.

Development of Prognostic and Predictive Variables in Breast Cancer: Personalized Approach Using Gene Expression Profiling Microarray

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Background: Despite a considerable decline in the mortality from breast cancer following systemic therapy, the biology of breast cancer remains poorly understood. Unfortunately, the routinely-used clinicopathologic variables fail to fully capture the biologic heterogeneity. As a result, many patients are overtreated whereas others may not receive the necessary therapy. Gene expression microarrays may provide more sophisticate information than conventional biomarkers in predicting disease outcome and response to a specific systemic therapy on an individual basis.
Methods: 1).To identify the molecular signature that predict pathologic complete response (pCR) to sequential treatment of paclitaxel, 5-fluorouracil, doxorubicin and cyclophosphamide (T/FAC) neoadjuvant chemotherapy, our group profiled 82 breast carcinomas and searched for the gene signature. Predicting accuracy of the signature was then validated on an independent set of 51 tumors. 2). To evaluate whether ER and HER2 status can be reliably measured from the comprehensive microarray data, we used gene expression data of 495 breast carcinomas to assess the correlation between ER and HER-2 mRNA levels and clinical status of these genes (as determined by immunohistochemical and/or fluorescence *in situ* hybridization). Data from 195 fine-needle aspiration (FNA) samples was used to define mRNA cutoff values and the accuracy of these cutoffs was assessed in two independent data sets: 123 FNA samples and 177 tissue specimens (ie, resected or core-needle biopsied tissues).
Results: 1). A 30-gene predictor of pCR was developed which could predict pCR with an overall accuracy of 76%. 2). ER and HER-2 mRNA levels correlated closely with routine receptor status measurements in all three data sets. Spearman's correlation coefficients ranged from 0.62 to 0.77. The defined ER mRNA cutoff identified ER-positive status with the overall accuracy of 88-96%; and the defined HER-2 mRNA cutoff identified HER-2-positive status with the overall accuracy of 89-93%.
Conclusions: 1). Although the gene signature may need to be refined and validated by large studies, this approach potentially allows us to tailor therapy based on expression microarray data. 2). ER and HER2 gene expression can be reliably measured from the comprehensive microarray data. Integration of ER and HER2 mRNA expression with multigene signatures from the same microarray data may refine and improve their predictive power. These findings may represent an important step towards personalized treatment.

Antibacterial RNA Silencing

GOOD L

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Background: New strategies are needed to combat antibacterial resistance. Our stategy is to target bacterial genes at the RNA level as a flexible genetics tool and possibly a new strategy for drug development. RNA silencing (or antisense) technologies can effectively target essential gene transcripts to block bacterial growth and sensitise bacteria to drugs that target proteins.
Methods: We have developed short synthetic antisense peptide nucleic acids (PNA). Cell uptake is enhanced using attached cell penetrating peptides (CPPs). In a second strategy, we have developed stabilised, expressed antisense RNAs. Therefore, we are able to silence bacterial RNA using both synthetic and expressed RNA strategies. Both RNA silencing tools have been used to silence established and putative antimicrobial drug targets. The effects of RNA silencing were assessed using Northern analysis, RT-PCR and by monitoring bacterial growth and survival.
Results: Synthetic antisense PNAs kill bacteria when targeted to stringently-required essential genes in *Esherichia coli*, *Staphylococcus aureus* and *Mycobacterium smegmatis*. These synthetic RNA silencers accummulate in target cells and display a long post antibiotic effect in the absence of cell lysis. It is also possible to activate cell death pathways by silencing antitoxin RNAs.
Expressed antisense RNAs are effective when modified to improve stability against endogenouse RNAse. We improved stability by using a paired termini design, where the 5´and 3´transcript are complementary and form a stable dsRNA duplex in cells. This design increases the abundance of antisense transcripts by extending transcript half life. Similar to synthetic RNA silencers, expressed RNA silencers are sufficiently effective to prevent bacaterial growth when targeted against growth essential genes.
Conclusions: Synthetic and expressed RNA silencers provide complimentary new tools for antimicrobial drug development and drug mechanism of action studies. Interestingly, partial silencing showed that established and putative antimicrobial drug targets differ significantly in their stringent requirement for growth. In other words, certain target genes are particularly sensitive to RNA silencing and may also provide more sensitive drug targets.

Medicinal effects of maca: Experimental data on reproduction, memory and learning

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Background: Peru has important tradition in the use of medicinal plants. *Lepidium meyenii* (maca), is a cruciferous cultivated plant growing over 4,000 m altitude in the central Andes of Peru traditionally used by its nutritional and supposed medicinal properties. The hypocotyl, the edible part of the plant is observed in different varieties (color). In the last years the interest for maca has been raised in many parts of the world. We have developed experimental and clinical studies to demonstrate the biological effects of this plant.
Methods: Clinical study was performed in 60 healthy adult men. Experimental studies were performed in rats (300 g BWt) and mice (30 g BW). Maca was boiled as traditionally used before administered. Red (RM) and Black (BM) variety were used in experimental studies. Maca was administered as boiled aqueous or hydroalcoholic extracts. Some experiments included the mixture of maca and another plant extract (UPCH). Toxicological studies were also performed. Data were assessed using parametric and non parametric statistics.
Results: In men gelatinized maca has favorable effects on energy, mood, decreasing anxiety, blood pressure, sexual desire, sperm production, sperm motility and semen volume without affecting sexual hormone levels. Experimental studies showed that BM increase sperm count and memory and learning in rats. Red maca reversed testosterone-induced prostatic hyperplasia and osteoporosis induced after ovariectomy in rodents. RM had more polyphenols and antioxidant activity than BM. The mixture BM+UPCH increased sperm count and reduced glycemia and serum Cholesterol in normal rats. Maca acute median lethal dose (LD50) for the oral route was >9 g/kg bw. Hydroalcoholic maca extract was not mutagenic *in vivo* and *in vitro* assays. Maca extract administered for 8 months to mice was not hepatotoxic rather than looks as hepatoprotective.
Conclusion: Gelatinized maca and extracts of black maca and red maca had acceptable safety profile and they are potential candidates as therapeutic agents.

	7 days
Control	83.46±5.87
BM (160 mg/Kg)	106.63±3.35*
UPCH 0.01 g/Kg + BM	107.75±6.07*
UPCH 0.1 g/Kg+BM	108.43±4.72*

Table 1. Epididymal sperm count (x10^6) after treatment with black maca (BM) alone or with UPCH (Plant extract). Data are mean ±SEM. Number of rats: 10 pergroup.*P<0.01

How did the MAGIC BULLETS drop the Knife out of the Surgeon's Hand.

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Background: Surgical drainage has been considered the treatment of choice in several abscess formations. We demonstrated that surgical drainage may be avoided by the use of antibiotics in certain pediatric surgical infections..
Methods: In two clinical studies among pediatric patients, we used needle aspiration combined with antibiotics as the treatment of suppurative cervical lymphadenitis and of perianal abscess referred for incision and drainage. In both cases, a local anesthetic ointment was applied for 1 hour before the aspiration and aspiration procedure was performed using a 19-gauge needle. In some cases, a conscious sedation with midazolam was used.
Results: Suppurative cervical lymphadenitis: This group included 35 children aged 4 months-13 years, mean 2.2 years referred for surgical drainage of node. Antibiotics used in most patients was cloxacillin. There were no complications. Patients were followed-up for 2-6 months. None required an open drainage of cervical abscess. A complete regression of the nodes was obtained in all patients within 21 days, with no relapse or scar formation.
Perianal abscess: Forty-seven infants (< 24 months of age) were treated by needle aspiration and antibiotics (gentamycin and metranidazole). A primary cure was obtained in 29 (61.7%) patients, and 16 (34%) children had an evolution toward a fistula in ano. Two infants had recurrent abscess successfully treated by the same way.
Conclusions: Needle aspiration combined with antibiotics seems to be an effective and safe treatment of suppurative cervical lymphadenitis and of perianal abscess. This modality which does not require general anesthesia may avoid the painful dressing change and drain removal, and does not lead to scar formation. We believe that our studies are additional steps for the continuous effort to replace invasive procedures by Magic Bullets.

Sulfadoxine- Pyrimethamine: Dead or Alive for Malaria Control?

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Background: Sulfadoxine-pyrimethamine (SP) has played an important role in malaria control across the world and is currently recommended for prevention as Intermittent Preventive Treatment for pregnant women (IPTp) and is being considered for prevention in infants (IPTi) in sub-Saharan Africa.
Methods: Review of the literature. Comparison of *in vivo* efficacy studies of SP and SP- IPTi protective efficacies with genetic markers of resistance against SP.
Findings: The molecular mechanism of drug resistance in *P. falciparum* was identified in the mid nineteen- nineties. Point mutations in the parasite folate pathway genes, namely in the parasite *dhfr* and *dhps* were associated with clinical failure of SP in uncomplicated malaria and conferred varying degrees of resistance. Identification of the point mutations and sequencing of microsatellites about the genes has allowed us to trace the spread of resistance from Asia to Africa. The proportion of isolates carrying these genes has risen to levels that some experts think heralds the end of SP. Although in areas of high resistance SP appears to have a continued effect for prophylaxis this is short lived and is not efficacious when the observation period is extended from 1 to 3 months.
Conclusion: Due to the patterns of genetic markers for drug resistance it is likely that SP has a short useful life in Eastern and Southern Africa, however in West Africa rates of mutations are much lower and SP may continue to be useful for some time.

Authors' disclosure statement
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Monoclonal Antibodies as Vaccine Adjuvants: From Potential to Protection

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Background: Targeting antigen (Ag) to Fc receptors (FcR) on Ag presenting cells enhances humoral and cellular immunity. Thus, we hypothesized that targeting inactivated *F. tularensis* (iFt) to FcR intranasally (i.n.), would enhance protection against mucosal challenge. We examined: **1)** the ability of anti-iFt monoclonal antibody (mAb) plus iFt, to enhance presentation of iFt Ag, **2)** the ability of mAb-iFt administered i.n. to enhance protection against i.n. challenge, **3)** the ability of an FcR-targeted subunit vaccine to protect against *S. pneumoniae*.
Methods: First, mouse macrophages and iFt Ag-specific T cells were combined with iFt or iFt plus anti-iFt mAb. Cells were incubated at 37°C, supernatants collected, and cytokine secretion measured. Second, mice were divided into three groups (5-6/group) and immunized i.n. with PBS, iFt, or mAb-iFt. Mice were immunized on day 0, boosted on day 21, challenged i.n. on day 35, and monitored 21 days for survival. Third, mice were divided into three groups consisting of wild-type (WT) mice immunized i.n. with PBS, WT mice immunized with *S. pneumoniae* Ag (PspA) in the form of anti-human FcγRI (hFcγRI)-PspA subunit vaccine, or transgenic mice expressing hFcγRI immunized with anti-hFcγRI-PspA. Mice were immunized on day 0 and 21 as above, and on day 35 sera were collected, or mice were challenged with *S. pneumoniae*, to measure Ab production or protection, respectively.
Results: Anti-iFt mAb plus iFt enhanced iFt presentation to Ag-specific T cells. When using mAb-iFt as an i.n. immunogen, increased protection (100%) was achieved compared to iFt alone (50%-65%). In addition, targeting PspA to hFcγRI i.n., in hFcγRI transgenic versus WT mice, enhanced *S. pneumoniae*-specific IgA and IgG production, and protection against i.n. challenge with *S. pneumoniae*.
Conclusions: The above studies demonstrate for the first time that targeting infectious disease Ag to FcR at a mucosal site is an effective strategy for enhancing protection against intracellular and extracellular mucosal pathogens. Furthermore, the versatility of this approach is demonstrated, in that mAb to an inactivated infectious agent can be used as an adjuvant, when a protective Ag has not been identified (*F. tularensis*). Alternatively, a subunit approach can be used when a protective Ag, such as PspA, has been identified (*S. pneumoniae*).

TLN-4601, a novel anticancer agent, inhibits Ras signaling through c-Raf degradation

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Background: TLN-4601 is a structurally novel farnesylated dibenzodiazepinone discovered though Thallion's DECIPHER® technology platform. The compound has demonstrated broad anti-tumor activity in vitro and in vivo. As TLN-4601 was identified through in vitro cytotoxic assays, its molecular target(s) was unknown at the time of discovery. Related to its farnesylated moiety, the effect of TLN-4601 on the Ras-MAPK signaling pathway was assessed.
Methods: Downstream Ras signaling events, Raf-1 and ERK1/2 phosphorylation, were evaluated by immunoblots in human breast (MCF7), glioma (U-87 MG), and prostate (PC-3) tumor cell lines. Cells were treated with 10 μM TLN-4601 in RPMI/0.1% FBS for 30 min, 1h, 4h and 6h, and subsequently exposed 10 min to EGF at 50 ng/mL. Cells were then lysed in ice-cold RIPA buffer extracts separated on SDS-PAGE. To study the effect of TLN-4601 on protein prenylation, exponentially growing cells were exposed to TLN-4601 or lovastatin (positive control) at 3, 10 and 30 μM for 24h. Cells were processed as above and immunoblots probed for RAP1A, HDJ2 and Ras.
Results: TLN-4601 exposure prevented EGF-induced phosphorylation of Raf-1 and ERK1/2. This effect was time and dose dependent with complete inhibition of protein phosphorylation within 6h at 10 μM. The inhibition of Ras-signaling was not mediated by inhibiton of protein prenylation, documented by the lack of effect of TLN-4601 on prenylation of HDJ2 or RAP1A, specific substrates of FTase and GGTase I, respectively. TLN-4601 treatment reduced Ras-GTP levels but did not inhibit EGFR, Raf-1, MEK or ERK1/2 kinase activities. Interestingly, we also noted that TLN-4601 induced Raf-1 proteosomal-dependent degradation.
Conclusions: Our data indicate that TLN-4601 inhibits the Ras-MAPK signaling pathway by two mechanisms: reducing Ras activation and depleting Raf-1 protein.

Radiological Detection of Dissolved Cocaine by Computed Tomography

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Background: Smuggling dissolved drugs, especially cocaine, in bottled liquids is an ongoing problem at borders. Common fluoroscopy of packages at the border cannot detect contaminated liquids. To find out if the liquids are contaminated, packages and bottles have to be opened, so that an immunologic test by the use of a drug test panel can be performed. Once opened, the cargo can not be delivered and tracked without arousing the suspicion of smugglers. The objective of our study was to develop a non-invasive MDCT (multi-detector computed tomography) screening method to detect cocaine-containing vessels that are hidden between uncontaminated ones in a shipment.
Methods: Studies were performed on three wine bottles containing cocaine solutions that were confiscated at the Swiss border. Reference values were obtained by scans of different sorts of commercially available wines and aqueous solutions of dissolved sugar. All bottles were scanned using MDCT, and data evaluation was performed by measuring the mean peak of Hounsfield units. To verify the method, simulated testing on twelve wine bottles including six contaminated bottles were performed.
Results: Using measurements of the mean peak of Hounsfield units, enables the detection of dissolved cocaine in wine bottles in a non-invasive and rapid fashion. Increasing opacity of the liquid corresponds well with the concentration of dissolved cocaine. Simulated testing showed, that it is possible to distinguish between cocaine-contaminated and uncontaminated wine bottles.
Conclusions: 1.) The described method is an efficacious screening method to detect cocaine-contaminated bottles that are hidden between untreated bottles in cargos. 2.) The non-invasive examination of cargo allows a questionable delivery to be tracked without arousing the suspicion of the smugglers.

Mast cell today.

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Mast cells are known to be among the most important effector immunocompetent cells participating in various biological responses. The description of the mast cell in connective tissue is ascribed to Ehrlich. Ehrlich made the important discovery that the mast cell stained well and specifically with certain dyes of the aniline family, the specific metachromatic staining reaction. Ehrlich also proposed the relation of mast cells with inflammation, blood vessels and neural tissue. Mast cell develop from progenitor cells that in turn arise from uncommited hemopoietic stem cells in the bone marrow, they undergo terminal differentiation in tissues. It was determined that IgE molecules have a high affinity for specific receptors on mast cells and that the reaction of cell-bound IgE molecules with multivalent antigens or divalent anti-IgE antibody induces the release of a variety of chemical mediators from the cells, that testify a central role for the mast cell in immunological reactions. More recently the role of mast cells in inflammatory disease and host defense was established. Following activation, these cells express mediators such as histamine, leukotrienes and prostaglandins, as well as proteases, and many cytokines and chemokines, pivotal to the genesis of an inflammatory response. Mast cells have been shown to play roles in allergic inflammation, and more recently, they have been shown to modulate coagulation cascades, host defense and tissue remodeling. Although mast cells were discovered long ago they are certainly no less interesting today and their history is far from complete

Augmenting Endogenous Nitric Oxide Production to Kill Multiresistant *Pseudomonas aeruginosa* in Cystic Fibrosis Lung Disease.

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Cystic fibrosis (CF) is among the most common of the fatal genetic diseases. CF affects multiple organs but lung disease is the major determinant of morbidity and mortality. Chronic inflammation and infection are the hallmarks of CF lung disease. **Nitric oxide** (NO) is involved in multiple aspects of lung biology including broncho-motor control and antimicrobial defense. Reduction of NO formation in CF is associated with airway obstruction and increased susceptibility to lung infections.
P. aeruginosa is capable of robust anaerobic growth by respiration using the NO metabolites nitrate (NO_3^-) or nitrite (NO_2^-) as terminal electron acceptors. Still, during anaerobic growth, *P. aeruginosa* must control the level of toxic NO by synthesis of protective NO reductase (NOR) and nitrite reductase (NIR).
As CF lung disease progresses mucoid *P. aeruginosa* strains emerge which are inherently resistant to antibiotics. The mechanisms behind the phenotypic switch to the mucoid form are incompletely understood, however the best characterized mechanism of mucoid conversion in CF isolates is via mutations in the *mucA* gene. NIR and NOR activity are remarkable low in *mucA* mutant *P. aeruginosa*, making this particular pathogen extraordinarily susceptible to NO-mediated killing. *MucA* mutant bacteria also have a markedly reduced capacity to remove NO generated aerobically from S-nitrosoglutathione. In addition, it was shown that 15 mM NO_2^- kills *mucA* mutant *P. aeruginosa* in CF airways at pH 6.5 under anaerobic conditions. In vitro experiments on the dose-effect relationship between NO_2^- concentration and killing of mucoid *P. aeruginosa* showed that the LD_{50} was approximately 3 mM NO_2^- after 24 hours. Previous studies demonstrated that overproduction of NO by anaerobic *P. aeruginosa* biofilms resulted in metabolic suicide of these bacteria, an event that was preventable by an NO scavenger. In vitro enhancement of antibiotic susceptibility of *P. aeruginosa* in biofilms had also been reported with L-arginine, NO_3^- or NO_2^- supplementation; presumably through an NO mediated mechanism.
Clinical and animal studies are currently ongoing to evaluate the therapeutic effects of L-arginine supplementation on pulmonary function and *Pseudomonas*-killing in the CF airways.

Delivery of specific targeted drugs into the cells by TAT-technology

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Background: The importance of drug delivery is pivotal in the wide area of pharmacological research and it has not been solved yet. The main goal of every drug delivery system is the delivery of a precise amount of a drug to the desired location in order to achive the necessary drug concentration in the targeted organ for effective treatment. Proteins and peptides are useful research and therapeutic tools, however their applications are limited because delivery to the desired location is not easily achiveable. Process of protein transduction, using TAT-technology, allows the delivery of drugs and genetic materials inside the cells. This process occurres in a rapid, concentration-dependent fashion that appears to be independent of receptors and transporters. It has a broad implications in experimental systems for regulating intracellular processes and has the potential to be used in the development of new therapeutic strategies for cancer, infectious diseases, and development of vaccines.
Hyper proliferation of cancer cells is associated with deregulation of cell cycle progression, which is driven by the activities of CDKs. A key regulator of their activities is protein p27. It has significant role in cancer progression and antitumor drug response.
Results: To examine p27 as specific target molecule and its role in tumor cells apoptosis, transduction of TAT-p27, TAT-ptp27 and TAT-N'p27 was performed. It was shown that different forms of TAT-p27 protein can modulate the cell cycle of cultured cell lines and induced apoptosis, depending on the concentration and type of the cells. Also was shown that different signal transduction pathways were involved in induction of apoptosis.
Conclusions: Extraxellular p27 could be use for induction of apoptosis in tumor cells. Protein transduction therefore could give an opportunity for delivering of drugs into cells with emphasis on specific target molecules.

The "antiinflammatory" 5-ASA, "immune-modulators" azathioprine, 6-MP, methotrexate & thalidomide and "immune-suppressants" Cyclosporine A, Rapamycin & Tacrolimus are all unsuspected "Magic Bullets" the inhibit *M. avium* subspecies *paratuberculosis* growth in culture

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Background: Without known mechanisms of action, "anti-inflammatories" (5-ASA,) "immune-modulators" (azathioprine, 6-MP, methotrexate & thalidomide) and "immune-suppressants" (Cyclosporine A, Rapamycin & Tacrolimus) are used to treat a variety of non-malignant "idiopathic" and "autoimmune" diseases. *M. avium* subspecies *paratuberculosis* (MAP) causes a chronic wasting diarrheal disease in ruminants called Johne's disease, that is evocative of human inflammatory bowel disease (IBD). MAP may be zoonotic. We hypothesized that, aside from their well-documented effects on eukaryotic cells, these agents may additionally be "Magic Bullets" that inhibit prokaryotes, particularly MAP. We herein report on the effects of these agents on MAP in culture.
Methods: We studied growth kinetics of four MAP strains (three human isolates, "Ben", "Dominic" & UCF-4) a bovine MAP isolate ATCC 19698 and three mycobacterial controls (*M. avium* & BCG,) using the radiometric $^{14}CO_2$ (Bactec®) system. Growth is quantified as arbitrary Growth Index (GI) units and inhibition as "percent decrease in cumulative GI" (%-ΔcGI.)
Results: (Most published at www.PLoSOne.org Thalidomide data Unpublished)
Our negative controls do not inhibit MAP. The test agents cause dose dependent inhibition of MAP. The most potent is methotrexate (89%-ΔcGI at 4µg/ml: "Dominic") followed by 6-MP, azathioprine, Cyclosporine A, Rapamycin, Tacrolimus and 5-ASA (46%-ΔcGI at 64µg/ml: ATCC 19698.) Thalidomide comprises two components. Phthalimide causes no inhibition, whereas the piperidine 2,6 dione moiety inhibits MAP (46%-ΔcGI at 64µg/ml: "Dominic".)
Conclusions: 1) We show in culture heretofore-undescribed inhibition of MAP growth by a variety of agents that are used, simply because of empirical efficacy, to treat several "inflammatory" and "autoimmune" diseases. 2) These data show that these agents effect prokaryotic, in addition to eukaryotic cells. 3) We conclude that, unknowingly, the medical profession has been treating MAP infections since 1942, when Nanna Svartz introduced sulfasalazine. 4) We posit that MAP may be responsible for multiple "inflammatory" and "autoimmune" diseases that have been empirically treated with these agents.

Authors' disclosure statement The author has submitted patents based on the hypotheses tested in these studies

Verapamil Reverts Acute Renal Functional Impairment Induced by Angiotensin II Converting Enzyme Inhibitors

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Background: Antihypertensive agents have been found effective in arresting glomerulosclerosis. Initially, it was thought that the healthy effect of these drugs was exclusively due to their hemodynamic effects. However, it has become clear that nonhemodynamic actions of these agents are an important component of their beneficial effects. Among thepharmac ological agents that may have a favorable
influence in the course of renal failure, angiotensin converting enzyme (ACE) inhibitors, and calcium channel blockers (CCB) have generated the most interest.
Angiotensin-converting enzyme inhibitors (ACEI) have proven to be effective drugs for the treatment of hypertension and represent a major therapeutic breakthrough in the management of hypertension and renal function preservation in diabetic and nondiabetic nephropathies. In some patients, ACEI may induce a rapid deterioration of renal function, assessed as an increase in serum creatinine (SCr), which can be reversed by withdrawing the drug.In these cases, maintaining the renal protection due to ACEI, instead of withdrawing these drugs could be desirable.
Calcium antagonists are a heterogeneous group of agents with diverse effects in terms of nephroprotection. Some of these differences relate to their effects on renal microcirculation. Dihydropyridine agents appear to act only on the afferent arteriole, increasing intraglomerular pressure, and albumin excretion rate. In contrast, nondihydropyridine agents like verapamil, may dilate efferent arterioles in addition to afferent arterioles and with normalization of the systemic blood pressure, verapamil may reduce intraglomerular pressure, and proteinuria. However, some other non hemodynamic protective effects of CCB could be explained by its capacity to inhibit the extracellular calcium influx, an important signal for the proliferative effect of mesangial cells mitogens, its influence in the decrease in mesangial entrapment of macromolecules and, possibly, its effect as free radical scavenger
Münter et al. describe that nephroprotective effects of ACEI/CCB combination can occur at doses, which do not significantly alter systemic blood pressure in the stroke-prone SHR. We have described that the combined therapy with these agents provide in the remnant kidney model a synergistic effect in preventing renal injury, independently of their effects on blood pressure.In a preliminary study, we have demonstrated in a small group of patients that nondihydropiridin (nonDHP) CCB are able to revert renal function reduction associated to ACEI treatment.
The main purpose of this study was to assess the efficacy and safety of low doses of verapamil (180 mg/day) added to the previous ACEI treatment for reverting decreased glomerular filtration rate observed in patients treated with ACEI. A secondary purpose was to test the ability of the fixed combination Verapamil-SR 180 mg plus Trandolapril 2 mg in attaining BP control and maintaining there renal function throughout the study.
Methods: This was a multicenter, nonrandomized, prospective, open study developed in five Spanish Hospitals. All patients were referred from the outpatients Departments of Internal Medicine and Nephrology. The Institutional Review Boards approved the study protocol and each patient entering the study signed a written informed consent.
Eligible patients presented a previous diagnosis of hypertension and an increase in SCr of 20% or 45 mmol/L from last values, in the course of ACEI treatment for more than four weeks. Exclusion criteria were renal insufficiency, defined as SCr >354 mmol/L (4 mg/dL), stroke, AMI in the last three months, unstable angina, cardiac failure, other causes of renal hypo-perfusion, or low volume syndrome such as dehydration, vomiting, diarrhea, laxative, and known hypersensitivity to verapamil.
In the selected group, a clinical evaluation was carried out, including clinical history, physical examination, BP measurement, ECG, and biochemistry (Visit 1). Patients fulfilling the inclusion/exclusion criteria were enrolled in the study.

[Regrettably not enough space for this long abstract.]

Molecular Modelling of Inhibitor-Kinase Interactions. 'Icy', Highly Polarized Water Molecules Can Tip the Relative Energy Balances of Competing Inhibitors.

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Background. Fak (Focal Adhesion Kinase) kinase is the target for the development of antitumor drugs. We perform molecular modelling computations in order to compare the relative energy balances for the binding to FAK kinase of two competing inhibitors in the pyrrolopyrimidine series, which were designed by the Novartis firm (Hao et al., *Bioorg. Med. Chem. Letts,* **2006***, 16*, 2809). The first, denoted as *16i*, has micromolar affinities while the second, *32*, has nanomolar affinities.
Methods. The protein-ligand interaction energies are computed by the SIBFA (Sum of Interactions Between Fragments Ab initio computed) procedure, an anisotropic, polarisable molecular mechanics procedure formulated an calibrated on the basis of ab initio Quantum Chemistry (Review paper: Gresh et al., *J. Chem. Theory. Comput*., **2007**, *3*, 960).
Results. The energy balances encompassing the contribution of continuum solvation favour *16i,* contrary to the experimental results. Including a limited number of five 'discrete', highly polarized, water molecules in the inhibitor-FAK recognition site results in an inversion of the energy balances, now in favour of *32*.
Conclusions. These findings imply that: 1) some structural, 'discrete' water molecules found in the recognition sites of protein can affect dramatically the comparative energy balances of structurally related, competing inhibitors. These water molecules undergo extremely large increases in their dipole moments amenable to quantum chemistry and polarisable molecular mechanics computations; 2) it is essential that the protein-inhibitor-water intermolecular interactions be computed with accurate potential energy functions, embodying the non-additive polarization contribution.

Cancer-testis antigens: effective molecules for developing successful immunotherapeutic strategies in the light of cancer complexity

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Background: Despite advances in our molecular knowledge, human cancer remains one of the major public health problems throughout the world. Although a number of cancer-testis (CT) antigens have been discovered and it has been suggested that they could be useful in the immunological treatment of cancer, the complexity of human beings leads us to reflect on the need to establish new criteria for validating their applicability. Aims: 1) To discuss cancer as a complex dynamical disease. 2) To discuss CT antigens as effective molecules for developing immunotherapeutic strategies in the light of cancer complexity.
Methods: This study investigate the immunolocalization of Sperm protein 17, a labeled CT antigen in multiple myeloma and ovarian carcinoma, in a panel of natural tissues of unrelated histologic origin and their tumoral counterparts. After deparaffination and rehydration, two-micrometers thick sections were placed in a bath for antigen retrieval, incubated with H_2O_2 to quench endogenous peroxidase activity, and then treated with monoclonal primary antibodies raised against Sp17 (BD Biosciences). Mouse IgG_1 was used as negative control. This was followed by incubation with the DAKO Envision system. 3,3′-diaminobenzidine tetrahydrochloride was used as a chromogen to yield brown reaction products. Countarstained slides were analyzed under a light microscope.
Results: Sp17 was found in human germinal cells of the testis (except for spermatogonia), and in the ciliated epithelia of the respiratory airways and in both the male and female reproductive systems. Sp17 has also been recognized in ovarian inclusion cysts, melanophages of cutaneous melanocytic lesions, as well as in a proportion of primary nervous system and liver tumors, a subset of esthesioneuroblastomas and a high number of pituitary adenomas.
Conclusions: 1) Sp17 is more widely distributed in the human body than originally thought. 2) The expression of CT antigens is mainly studied at the level of gene expression and gene level measurement by RT-PCR analysis and the quantitative RT-PCR technology. However, the information provided by these approaches is limited by the fact that the phenomena observed at each level of anatomical organization have properties that do not exist at a lower or higher level. 3) A multidisciplinary system-level approach, which takes into account the human being as a complex hierarchical system, provides a different way of investigating human cancer, thus promising a more widely applicability of CT antigens for developing effective immunotherapeutic strategies.

Ascorbic acid and dietary selenium bioavailability in rats

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Background: Vitamin C is a nutrient that may affect the bioavailability of both trace (copper and iron) and undesirable (lead, mercury, and cadmium) elements. It is also quite possible that vitamin C may interfere with other trace elements. For example it is suggested that vitamin C may reduce dietary selenium to an elementary form that cannot be utilized by the body. Taking into account a very narrow range of the save selenium intake the purpose of this study was to evaluate if moderate dietary vitamin C supplements affect selenium retention in selected organs of rats
Methods: Studies involved 135 male Wistar rats, 212 ± 11 assigned into three groups each of 45 animals: group 1 (controls), and groups 2 and 3 exposed to 1g/L and 2 g/L of vitamin C via drinking water, respectively. All rats received traces of sodium selenite (labeled with selenium-75) by a stomach tube for 28 days. Selenium-75 activity was measured in the duodenum, blood, liver, kidneys, spleen, muscles, heart, brain, and testicles within 28 days postdosing. Results were compared using Student's t-test at $P < O.05$.
Results: No differences were noted in tap water (about 29 mL/rat/d) and feed intake, organ to body ratio, and body weight gains among the groups tested. The retention of selenium –75 in selected organs was shown as the AUC values. In groups 2 and 3 the AUC values were compared to those in the controls that were considered as 100 per cent. Data were analyzed statistically using Student's *t*-test at $P<0.05$.

Vit. C in water	Blood AUC	Liver AUC	Kidneys AUC	Skeletal muscles AUC	Testicles AUC
0 g/L	100 (in %)	100 (in %)	100% (in %)	100% (in %)	100% (in %)
1 g/L	151*	152*	131*	124	134*
2 g/L	121	132*	125*	110	119

Conclusions: 1) Diet supplemented with moderate doses of vitamin C may enhance selenium retention in the organs especially those revealing a high rate of selenium metabolism. 2) The ability to raise selenium retention seems to be inversely related to increasing doses of vitamin C when a dosage is higher than 1 mg/L. 3) It is suggested that vitamin C may improve selenium bioavailability when this element consumption is low or not adequate; however, it should be stressed that too high intake of vitamin C may be undesirable when selenium intake sufficient.

Acetazolamide Inhibits Electrogenic Sodium Bicarbonate Flux through kNBC1. Molecular Mechanisms and Computer Simulations.

GROSS E

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Background: The HCO3- : Na+ cotransport stoichiometry of the electrogenic sodium bicarbonate cotransporter kNBC1 determines the reversal potential (E(rev)) and thus the net direction of transport of these ions through the cotransporter and thus across the cell membrane (i.e. efflux or influx). Phosphorylation of kNBC1-Ser(982) in the carboxy-terminus of kNBC1, by cAMP-protein kinase A (PKA), shifts the stoichiometry from 3 : 1 to 2 : 1. Downstream of Ser(982) in kNBC1 is a D986NDD motif. A homologous motif (D887ADD) in the carboxy-terminus of the anion exchanger AE1 binds to carbonic anhydrase II (CAII). We thus studied the binding of kNBC1 to CAII and the role of the D986NDD motif in this protein-protein interaction.
Methods: We used isothermal titration calorimetry to measure the binding constant of CAII to kNB1 and Ussing chamber electrophysiology apparatus to measure the electogenic flux of sodium and biacrboanet through the cotransporter.
Results: In isothermal titration calorimetry experiments, CAII was found to bind to wt kNBC1-Ct with a K(D) of 160 +/- 10 nM. Acetazolamide inhibited the short-circuit current through the cotransporter by 65 +/- 6 % when the latter operated in the 3 : 1 mode, but had no effect on the current in the 2 : 1 mode.
Conclusions: We propose a model in which CAII, when bound to kNBC1, builds a high local concentration of bicarbonate in the vicinity of the cotransporter's anionic binding site. Phosphorylation of kNBC1 by PKA removes CAII and as a result lowers local bicarbonate concentration and shifts the stoichiometry to 2:1. This model is also supported by computer simulations with a six-state transport binding scheme and electric field modulated binding constants and membrane translocation steps.

Increased repolarization reserve as a new anti-arrhythmic principle

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Background: A healthy human heart will beat approximately 3.000.000.000 times during a normal lifespan without any disturbances. Any electrical deviation from this regular pathern is termed an arrhythmia. Arrhythmias can result in anything from minor palpations to sudden cardiac death. A number of arrhythmias are due to malfunction of a cardiac ion channel named HERG1. This channel is essential for appropriate repolarisation of the cardiac action potential. It is well known that unintended inhibition of the HERG channel is pro-arrhythmic. We have therefore developed a new concept of HERG channel activation an investigated the anti-arrhythmic properties of such activators.
Methods: The experimental approach is translational. Patch-clamp experiments have been conducted applying native cardiomyocytes or by using heterologous expression systems in oocytes and mammalian cells of the HERG channel. In addition, ex vivo Langendorff experiments and in vivo studies in both conscious and anaestezied animals have been conducted.
Results: A number of anti-arrhythmic properties was demonstrated for the HERG channel activators. In native cardiomyocytes action potential was abbreviated and post-repolarisation refractory period was increased significantly. Further HERG channel activation rendered the cardiomyocytes more resitance towards early-afterdepolarisations (EAD's). In intact hearts investigated in Langendorff set-up extrasystolis could be prevented by HERG channel activation and a tendency towards less dispersion of the length of action potentials was observed. In in vivo studies HERG channel activation could prevent drug induced prolongation of the QT interval and significantly reduce the incidence of extrasystolis and ventricular fibrillations.
Conclusions: In conclusion we believe it is demonstrated that under certain circumstances, activation of the cardiac HERG channel can be a new antyi.-arrhythmic principle

Infusion Monitoring of Anesthetic Drugs: Propofol in Respiratory Gas

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Background: The continuous monitoring of propofol concentration in breathing gas by an electrochemical sensor (ELCH) allows a determination of changes in plasma concentration. The following animal study should test the hypothesis whether a bolus of propofol induces time related changes in breathing gas, that can be compared to the changes in calculated plasma and effect site propofol concentration.
Methods: After the approval of the regional authority 8 pigs in a healthy condition were investigated. After propofol free induction propofol was applied as a bolus (4 mg/ kg body weight) at 0 and 30 minutes and with a continuous infusion of propofol (9,6 mg/ kg body weight x h) simultaneously. The time to peak after the propofol bolus was determined for the concentration of propofol in breathing gas by ELCH and in plasma and effect site (MARSH –model). The non-parametric Mann – Whitney U-test was used to compare them (*: p <5%).
Results: In 4 of the 8 animals the first and in all 8 animals the second bolus was detected as a peak of the propofol concentration in breathing gas measured by ELCH (see table 1).

Treatment	Number of animals (1)	t (breathing gas) (s)	t (plasma) (s)	t (effect site) (s)
Bolus 0 min	4 of 8	321	25	115
Bolus 30 min	8 of 8	354 (*)	20	110

The ELCH resolved a bolus as a peak in breathing gas concentration after propofol has been given already for 30 min as a continuous infusion. At the beginning of a propofol anaesthesia a bolus was indicated as a concentration change and not as a peak in 50% of the animals.
Conclusions: Differences in time to peak in breathing gas compared to effect site and plasma illustrate how fast propofol is distributed to different compartments. Ongoing investigations using other methods for real time measurement of propofol in breathing gas will help to further separate possible influences of the measured appearance of propofol in breathing gas due to equipment from influences due to physiology.
Authors' disclosure statement:
There are financial obligations between the authors and the Drägerwerk AG Co. KGaA, Germany

Treatment of ovarian cancer cells with drug combinations targeting ErbB receptor tyrosine kinases and fatty acid synthase

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Treatment of ovarian cancer (OC) is still suboptimal necessitating the search for novel therapies. In normal tissue, the key lipogenic enzyme fatty acid synthase (FASN) converts dietary carbohydrates to triglycerides, whereas in cancer, FASN represents a metabolic oncogene and produces phospholipids for membrane microdomains (lipid rafts) that accommodate clusters of receptor tyrosine kinases including Epidermal Growth Factor Receptor (EGFR, ErbB1) and ErbB2 (HER-2/neu) thus setting the stage for signal initiation. Importantly, both FASN and ErbBs are overexpressed in tumors including OC and represent drugable targets. Recent data suggest a link between FAS and ErbB2 in breast cancer. In OC, the relationship between FAS and ErbB is still elusive. Therefore, we examined the effect of FAS and ErbB inhibition on A2780 ovarian cancer cells (OCC). A FASN inhibitor (C75) and 2 irreversible ErbB inhibitors (EKB-569, Wyeth; CI-1033, Pfizer) inhibit growth of OCC (MTT assay - IC50: C75=22 µM; EKB-569=5,1 µM; CI-1033=3,7 µM). Interestingly, C75 synergizes with EKB-569 or CI-1033 in cell growth inhibition ($p<0.01$) suggesting cooperation between FAS and ErbB pathways during OCC growth. RT-PCR, real-time analysis and Western blotting revealed that C75 slowly and concordantly reduces EGFR mRNA, protein and activity in OCC. Thus, C75 silences EGFR gene expression at transcriptional levels without directly affecting EGFR signaling. C75 caused deprivation of overall and phosphorylated ErbB2 protein, but failed to diminish ErbB2 mRNA. Although C75 post-transcriptionally represses ErbB2, it does not directly disrupt ErbB2 activity. C75 also caused shut-down of FAS mRNA and protein. On the other hand, EKB-569 abolishes EGFR and ErbB2 protein expression and phosphorylation, but only weakly depresses mRNA levels. Strikingly, EKB-569 also represses FAS mRNA and protein. CI-1033 also failed to affect EGFR and ErbB2 transcript levels, but compromised EGFR activity (but not EGFR protein expression) and ErbB2 protein expression and function. Generally, CI-1033 reduced ErbB function rather than ErbB protein expression. Moreover, CI-1033 correspondingly down-regulated FAS mRNA and protein. Our data indicate that ErbB and FAS pathways mutually interact with each other in OCC. Thus, interference with the FAS and the ErbB systems effectively abrogates their oncogenic activities and may be exploited for OCC treatment. Supported by ‚Initiative Krebsforschung', Vienna, Austria.

The PI3K/AKT pathway determines EGFR/HER/ErbB drug efficacy in breast cancer cells.

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ErbB transmembrane proteins belong to the family of tyrosine kinase receptors. Four members have been described: ErbB1 (EGFR), ErbB2, ErbB3, and ErbB4. ErbB1 and 2 are overexpressed/hyperactivated in many tumors, including ovarian and breast cancer. They stimulate carcinogenesis and malignant progression, and confer unfavorable prognosis. Clinical success has recently been obtained by targeting ErbB2 in ErbB2+ breast cancer. However, only 30% of ErbB2+ breast cancers respond to targeted ErbB2 blockade and most of the responders develop secondary resistance. The situation is even worse, when ovarian cancer is considered. Unfortunately, predictive markers for assessing ErbB inhibitor sensitivity/resistance are still widely lacking. Using MTT assay and Western blotting we examined the effects of the novel irreversible ErbB inhibitor pelitinib (EKB-569, Wyeth) on the growth activity and on ErbB-triggered signaling in 11 human breast and 11 human ovarian cancer cell lines. SKBR3 and T47D were identified as most sensitive and most resistant breast cancer cell lines, respectively. In contrast, the sensitivity of the ovarian cancer cell lines did not vary as much. Interestingly, the antiproliferative activity of the drug did not correlate with EGFR and ErbB2 protein levels. Moreover, drug-dependent inhibition of EGFR, of ErbB2 and of ERK1/2 phosphorylation was seen in both pelitinib-sensitive and pelitinib-resistant cells indicating that inhibition of ERK1/2 downstream signaling is not sufficient for drug-dependent growth arrest. In contrast, phosphorylation of ErbB3 at Tyr1289, of AKT at Ser473 and at Thr308, and of GSK3beta at Ser9 was blocked only in the sensitive, but not in the resistant cells. Moreover, ectopic expression of constitutively active AKT induced resistance to pelitinib in SKBR-3 cells. Conversely, pelitinib rapidly induced phosphorylation of PTEN at Ser380 in sensitive, but not in resistant cells. Taken together, our data suggest that ErbB3/PI3K/AKT, but not ERK1/2 signaling plays crucial roles in determining sensitivity/resistance of the cells against the irreversible dual EGFR/ErbB2 inhibitor pelitinib. Therefore, we propose that drug-mediated downregulation of phospho-AKT and phospho-ErbB3 levels might be useful surrogate markers for ErbB drug efficacy in breast cancer. Supported by ‚Initiative Krebsforschung', Vienna, Austria.

Expression of Fas on human T lymphocytes under stimulation with *Borrelia burgdorferi* sensu lato.

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Background: Pathogenesis of late Lyme disease (LD) remains controversial, and the possibility of 1) autoimmune complications after elimination of Borrelia burgdorferi sensu lato (B.b.) infection and 2) impaired immunity preventing pathogen elimination, has been proposed. As apoptosis of activated T lymphocytes, on the Fas receptor pathway, is essential in control of inflammatory/immune response, its abnormalities in contact with B.b. could lead to both of these conditions.
Methods: We measured Fas expression on peripheral blood CD3+ and CD4+ lymphocytes under stimulation with B. burgdorferi s.l. Peripheral blood mononuclear cells were derived from 23 patients with late LD (18 with Lyme arthritis - LA and 5 with neuroborreliosis - NB) and 13 healthy persons (controls, C). Cells were incubated for 48 hours without stimulation (neg.) or with the suspension of inactivated Borrelia burgdorferi spirochetes: B.afzelii VS 46110, B.garinii 20047 or B.burgdorferi sensu stricte B-31, with bacteria to PBMC ratio 10:1, as antigenic stimulation. Fas expression on CD3+ (for all studied subjects) and CD4+ (for 8 subjects in LA, 4 in NB and 8 in C group) cells was measured by flow cytometry with FITC-labeled anti-Fas monoclonal antibody.
Results: Expression of Fas on CD3+ and CD4+ cells increased with age. When corrected for age, there was no difference between between LD and C groups. In LD patients, Fas expression did not depend on clinical form and duration of the disease. Median Fas expression increased significantly ($p < 0,05$) under stimulation with any of the B.b. strains: on CD3+ cells from 34% to 41-42% and on CD4+ from 23% to 24-26%. For CD3+ the increase was comparable in LA, NB and C groups, while for CD4+ cells it was signifiacant only in LA group.
Conclusions: Exposition to B.b causes moderate, but significant, increase of Fas death receptor on CD3+ and CD4+ peripheral blood limfocytes, which may render these cells more susceptible to apoptosis. Possible role of this phenomenon in pathogenesis of LD requires further study.

Dopamine transporter as the target and carrier of illicit and therapeutic drugs – PK/PD approaches to develop MAGIC BULLETS for cocaine abuse

GU H

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Background: Cocaine is a powerful psychostimulant and an addictive drug of abuse. There are three known high-affinity targets for cocaine: the dopamine transporter (DAT), the serotonin transporter (SERT), and the norepinephrine transporter (NET). Decades of studies support the dopamine (DA) hypothesis that the blockade of DAT and the subsequent increase in extracellular DA primarily mediate cocaine reward and reinforcement. Contrary to expectations, DAT knockout mice (DAT-KO), and SERT or NET knockout mice still display the rewarding property of cocaine. These studies indicate that none of these transporters are required for the cocaine effects and led to the re-evaluation of the DA hypothesis and the proposal of redundant reward pathways. However, the knockout mice have very significant adaptive changes during development to compensate for the complete absence of a critical protein, which might have altered how cocaine produces its effects in these mice.
Methods: To study the role of DAT in cocaine reward, we have engineered a functional but cocaine-insensitive mutant of DAT and generated a knock-in mouse line carrying this DAT mutant (DAT-CI mice). Normal doses of cocaine still block SERT and NET but have little effect on DAT in these mice which provide a unique tool to study the role of DAT in mediating cocaine effects. We also used an Adenyl Associate Virus (AAV) vector to re-introduce the wild type DAT back into specific brain regions of DAT-CI mice to study whether cocaine responses can be restored and what brain regions are critical for which cocaine responses.
Results: In DAT-CI mice, cocaine did not elevate extracellular DA in the nucleus accumbens (NAc), cocaine did not stimulate locomotor activity but suppressed it, and cocaine failed to produce reward as measured by conditioned place preference and by drug self administration. In contrast, amphetamine, another psychostimulant, was able to stimulate locomotor activity and produce reward, indicating that the reward system functioned well in these mice. In addition, re-introducing wild type DAT back into the brains of fully developed DAT-CI mice restored the ability of cocaine to stimulate locomotor activity and to produce conditioned place preference.
Conclusions: Our results indicate that cocaine blockade of DAT is required for the stimulating and rewarding effects of cocaine in mice with a functional DAT. While cocaine can produce reward in mice without DAT but it is through a mechanism different from that in normal mice. Therefore, under some abnormal conditions, possibly when the DA system is defective, cocaine may produce reward by interacting with targets other than DAT. Furthermore, our results suggest that drugs that prevent cocaine binding to DAT should reduce the stimulating and rewarding effects of cocaine and thus may be effective in treating cocaine addiction.
MAGIC BULLETS development: We are now collaborating with other investigators to screen large chemical libraries for **MAGIC** compounds that prevent cocaine binding while still allow transport. We are also working on the natural esterases responsible for cocaine degradation to develop a **MAGIC** enzyme that significantly reduce the bioavailability of cocaine.

Drugs Legeslation and Regulation in Pakistan

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Objectives: The present study focused on data regarding the current situation of drugs regulation in Pakistan, its developmental history and other related issues.
Introduction: Pakistan is committed to the goal of health for all, inspired by the principle of social equity. To achieve this, the government is taking all possible measures in general, and drugs in, particular. National drugs policy emphasizes to ensure the availability, efficacy, safety and quality of the essential drugs in affordable Prices. Essential drugs are those which help in combating diseases, maintaining and improving the health status of the population. Government is also emphasizing to ensure the availability and acceptability of drugs in the country, to protect the public from hazards of substantial and unsafe drugs. To achieve this goal they are trying to develop skillful persons in the drugs manufacture fields, so they developed an operational and applied research in the field of pharmaceuticals.
Materials: Data about the drugs regulation in Pakistan was collected using the internet database and other published materials. Wherever needed, personal interviews with the concerned personals or other communication means were used for data collection. The information collected were arranged and compiled in a proper sequence.
Results & Discussion: Pakistan has a fairly modern legislation namely the Drugs Act 1976. Under this act rules have been formed on the various aspect of drugs control. It provides a system of licensing of all manufacturing houses and registration of the finished drugs to ensure efficacy, safety and quality of the marketing drugs. A board has been established for the export, import and quality control on federal and provincial bases. The quality is controlled by inspectors and laboratory services. The laws have been considered to be fairly modern in favor of public safety. From production and marketing view point, the government extends full support to the drug producers and drugs dealers. Incentives are provided to the hospitals and the local industry. About 80% of the drugs are locally prepared by 285 companies including 25 multinational and trying for self sufficiency. To ensure the quality, 8 inspectors on federal whereas 81 inspectors are working in provincial setup.
Conclusion & Recommendations: The traditional system of medicines is not properly regulated and hence efforts are made to regulate it through law with a view to their rationalization, to improve standard and for the protection of the public from hazards. Comprehensive public information should be launched to enhance under standing and acceptance of the essential drugs concept by the health professionals. For the selection of essential drugs, the ease in availability of essential and genuine drugs should be ensured by the government.

Genetic Polymorphisms of Cytochrome P450 and Risk on Prostate and Bladder Cancer

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Background: Human cytochrome P450 (CYPs) enzymes are oxidative enzymes responsible for the metabolic activation of many precarcinogens and participate in the activation and inactivation of anticancer drugs. Genetic differences of the detoxification system may cause an increase in susceptibility to environmentally induced bladder cancer. Genetic differences of the detoxification system may cause an increase in susceptibility to environmentally induced bladder cancer and prostate cancer. Aims: 1) To investigate the potential association between the cytochrome p450 1A2 (*CYP1A2*) 734 C>A and cytochrome p450 2D6 (*CYP2D6) 1934 G>A* gene polymorphisms and the risk of bladder cancer in a Turkish population. 2) To investigate the relationship of prostate cancer (PCa) with genetic polymorphism of 17-hydroxylase (CYP17) (-34 T/C) and CYP1A1 (T/C) genes in a Turkish population.
Methods: This study included 135 bladder cancer patients and 128 age and sex matched cancer-free controls; 148 prostate cancer patients, 136 benign prostatic hyperplasia patients, and 102 healthy individuals as controls. The polymorphisms were analyzed using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) assay. Genotype and allele frequencies and their associations with bladder and prostate cancer risk, demographic factors, smoking status, and tumor stage were investigated.
Results: 1) There was no association between studied polymorphisms of *CYP1A2*, and *CYP2D6* genes and bladder cancer. 2) No statistically significant association was observed between smoking status of the patients and any of the polymorphisms studied. 3) We have observed that there is statistically significant association between the smokers with the *CYP1A2* CC genotype and the bladder cancer risk but not with the non-smoker subjects with CC genotype (OR=2.55; %95CI, 1.030-6.316). 4) No association was observed between prostate cancer and 17 hydroxylase gene polymorphism. 5) There was also an association between 17 hydroxylase polymorphism and benign prostatic hyperplasia (*P*=0.004).
Conclusions: These data demonstrate that cytochrome P450 enzymes polymorphisms studied may not be associated with bladder cancer and CaP population studied. In addition, the results suggest that the genotypes of *CYP1A2* polymorphism may be associated with increased risk of bladder cancer in smokers.

Keywords: CYP450 polymorphisms, bladder cancer, prostate cancer

Cholinesterase Inhibitors and NMDA Receptor Antagonists in Alzheimer's Disease and Pesticide Poisoning.

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Both Alzheimer's disease (AD) and anticholinesterase pesticide (organophosphate and carbamate) poisoning are characterized by alterations in the cholinergic and glutamatergic systems. In addition to neurodegeneration, common biochemical mechanisms in the discrete brain areas of concern, cortex and hippocampus, involve up-regulation of NMDA receptors, excess free radical generation, oxidative/nitrosative stress, depletion of enzyme-based antioxidant system and high- energy phosphates. While AD is characterized by a marked decline in acetylcholine (ACh) due to the loss of cholinergic neurons (as much as 90%), immediate reaction in the organophosphate (OP) and carbamate (CM) poisoning is acetylcholinesterase (AChE) inhibition, followed by ACh accumulation. Currently, donepezil (Aricept), rivastigmine (Exelon), and galantamine (Reminyl) are the three reversible AChE inhibitors most commonly indicated in AD patients to elevate the levels of ACh, which is required to sustain precious memory. However, these AChE inhibitors are indicated only in mild to moderate AD, and patients often become refractory to the beneficial effects after a year of treatment. Our findings revealed that a combination therapy with donepezil (0.75 or 1.5 mg/kg, ip) or rivastigmine (0.35 or 0.7 mg/kg, ip) and an NMDA receptor antagonist like memantine (10 mg/kg, ip) or neramexane (3.1, 6.2, or 12.4 mg/kg, ip), has no interaction at the AChE level and may provide the optimal therapeutic effect. Our study with cholinesterase inhibitors and NMDA receptor antagonist in the animal model revealed that pretreatment with memantine (18 mg/kg, sc) in combination with atropine sulfate (16 mg/kg, sc) provides remarkable protection against an OP compound DFP (1.5 mg/kg, sc) or a carbamate compound carbofuran (1.5 mg/kg, sc) induced behavioral toxicity, AChE inhibition and decline of high-energy phosphates. In addition, these antidotal pretreatments suppressed DFP or carbofuran induced increase in markers of oxidative (F_2-isoprostane and F_4-neuroprostane) and nitrosative (citrulline) stress, inflammation (PGE_2) and morphological changes in the dendritic system of pyramidal neurons from CA1 hippocampal area. It can be concluded that the lack of interaction between AChE inhibitors (donepezil or rivastigmine) and NMDA receptor antagonists (memantine or neramexane) at the AChE level benefits in treatment of AD, while interaction benefits in prophylaxis and treatment of OP/CM poisoning.

Antileishmanial Efficacy of Amphotericin B bearing Emulsomes against Experimental Visceral Leishmaniasis

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Background: Because lipid particles are phagocytized by the reticuloendothelial system, lipid associated amphotericin B (AmB) should be concentrated in infected macrophages (MQs) of liver and spleen and be very effective against visceral leishmaniasis (VL). Aims: 1) To develop emulsomes for effective and site specific localization of AmB. 2) Passive and active targeting of AmB inside the MQs. 3) To treat the VL.
Methods: AmB loaded plain emulsomes (PE) and O-palmitoyl mannan coated emulsomes (CE) were prepared by cast film method followed by homogenization. Organ distribution study included 72 male albino rats (weight 150-200g, 18 rats per treatment, one control group). AmB-deoxycholate (AD), PE and CE containing doses equivalent to 1mg/kg of AmB were administered i.v. to different groups. The antileishmanial activity of AD, PE and CE was tested *in vitro* at different drug doses (0.03, 0.08, 0.13 and 0.2 µg/ml) in *Leishmania donovani* infected MQ-amastigote system (J774A.1 cells). *L. donovani* infected hamsters (weight 80-100 g) harboring 38-40 amastigotes/100 MQ nuclei were distributed (6 in each group, total 36, 3 control groups) for drug (equivalent to 0.5 mg/kg) treatment intracardially. Each experiment was run for 3 times.
Results: The *in vitro* antileishmanial activity showed higher efficacy of CE (92.27±5.7% parasite inhibition, PI) over PE (82.57±5.4% PI) and AD (65.71±5.1% PI) ($p<0.05$ for AD vs PE; $p<0.01$ for AD vs CE) [data at dose of 0.08 µg/ml]. The extent of accumulation of AmB in MQs rich organs, liver and spleen was significantly high from developed systems (54.47±3.4 and 13.48±0.91% from PE; 59.67±4.2 and 14.12±0.9% from CE after 24 h) when compared against AD (15.74±1.1 and 1.14±0.08% after 24 h). Formulation CE eliminated *L. donovani* amastigotes within splenic MQs more efficiently (73.7±6.7% PI) than PE (51.7±5.4% PI) ($p<0.01$) or AD (30.4±4.8% PI) ($p<0.001$).
Conclusions: 1) The proposed systems showed excellent potential for MQ targeting as shown by drug levels in liver and spleen. 2) The formulations could significantly modify the pharmacokinetics of AmB as compared to AD, providing prolonged action at comparatively low drug doses thereby reducing the toxicity problems like nephrotoxicity, cardiac arrhythmia etc.

Ciprofloxacin Induces Oxidative Stress and Exerts Biphasic Cytotoxicity in Primary Culture of Rat Astrocytes and Human Fibroblast Cells.

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Background: Mechanism underlying adverse effects of ciprofloxacin (CPFX) is still not well known. Aims: The possible cytotoxic and oxidative stress inducing effects of CPFX were investigated on primary cultures of rat astrocytes and human fibroblast cells.
Methods: The cultured cells were incubated with various concentrations of CPFX (0.5-300 mg/l for astrocytes, n= 4; 5-150 mg/l for fibroblast cells, n= 3), and cytotoxicity was determined by neutral red (NR) and/or MTT assays. Oxidative stress induction possibility by the drug were assayed measuring lipid peroxidation (LP) and total glutathione (GSH) levels, and activity of catalase (Cat) in both cell types. Superoxide dismutases and glutathione peroxidase activities were determined only in fibroblast cells.
Results: 1) Survival profile of astrocyte cells was biphasic in NR assay: While CPFX did not cause any alteration at any concentration for 7 h, ¡Ü50 mg/l concentrations induced significant ($p< 0.05$) cell proliferation in 24, 48, 72, and 96 h. However, cell proliferation gradually decreased at higher concentrations, and 200 and 300 mg/l of CPFX was found to be significantly ($p< 0.05$) cytotoxic at all time periods. With MTT assay, no alteration was noted for 7 h. But, viability decreased with ¡Ý 50 mg/l CPFX exposure in all other time periods. Cell proliferation was only seen in 24 h with 0.5 and 5 mg/l CPFX. A significant enhancement of LP was observed with the 300 mg/l of the drug, but GSH, and Cat content of cells did not change. 2) Cytotoxicity was not observed with 5-150 mg/l CPFX when the human fibroblast cells were incubated for 24 h. 5-12.5 mg/l of CPFX increased the cell growth in all incubation periods tested. Marked decreases in the viability of fibroblasts were observed at 50 and 75 mg/l, and ¡Ý 50 mg/l, following 48 and 72 h exposure, respectively ($p< 0.05$). An induction of LP and a marked decrease in intracellular GSH were observed following incubation of cells with 75 mg/l CPFX for 48 h. Vitamin E pretreatment provided protection in both cell types.
Conclusions: 1) CPFX-induced cytotoxicity is related to oxidative stress. 2) The hormetic-like biphasic effects of CPFX possibly resulted from the complex dosedependent relationships between reactive oxygen species, cell proliferation, and cell viability.

Transient Activation of the Small GTPase Rap1 is Functionally Required for the Regulation of Breast Cancer Cell Motile Responses to IGF-I

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Background: The Ras family GTPase Rap1 is an essential regulator for adhesion receptors, actin dynamics, and cell migration. The mechanisms by which Rap1 controls motile responses in the epithelial cells remain unknown. In fact, current view is that activation of Rap1 tightens cell-cell junctions and thus may have anti-metastatic effects in cancer. Normal and cancer cells share common, and perhaps evolutionarily conserved, motility machinery activated by insulin-like growth factor I (IGF-I).
Methods: We used an *in vivo* tool, a fusion of enhanced green fluorescent protein (EGFP) to the Rap1 binding domain (RBD) of RalGDS that is recruited specifically to the sites of increased Rap1 activity. By confocal laser-scanning microscopy, we monitored dynamics of EGFP-RBD-RalGDS; by time-lapse video microscopy we tracked localization of the EGFP-Rap1 molecules in live cancer cells. By image-based quantitative immunohistochemistry, we compared 23 normal/benign (N/B) and 32 invasive breast cancer (InvBC) surgical specimens.
Results: We report that in human breast cancer cells a rapid enhancement and a subsequent gradual decrease of Rap1 activity induced by IGF-I promote cell motile responses: breakdown of cell-cell contacts and formation of lamellipodia. This transient activation of Rap1 requires the kinase activity of the IGF-I receptor (IGF-IR) and receptor internalization. Time-lapse video microscopy in live cells confirmed a disappearance of EGFP-tagged WTRap1 and constitutively active V12Rap1 from cell-cell contacts. We also found accumulation of the active endogenous Rap1 in lamellipodia of IGF-I-stimulated cells, whereas selective blocking of IGF-I-induced Rap1 activation by over-expressed RapGAP restrained lamellipodia formation. Quantitative analysis of surgical specimens revealed significantly higher protein levels of Rap1 (p=6.23E-04) and IGF-IR (p=4.32E-07) in InvBC compared with N/B breast tissue.
Conclusions: 1) Presented data provide experimental evidence that transient activation of Rap1 by IGF-IR-mediated mechanism promotes cancer cell motile responses and that active Rap1 does not prevent cell-cell separation induced by IGF-I. 2) Screens of surgical specimens show significant over-expression of IGF-IR and its target Rap1 in infiltrating carcinoma of the breast.

Magic Microspheres for Joint Treatment. Why Doxycycline?

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Background: Septic arthritis is common in large animals and local therapy has proven to be favorable over systemic antibiotic treatment. Doxycycline (DOX) was thought to encompass the spectrum against bacteria usually isolated from bovine and equine joints and to provide in addition a chondroprotective effect. DOX was microencapsulated into poly(lactide-co-glycolide) (PLGA) microspheres for prolonged release over 10 to 15 days, which should afford a single injection for a fortnight's treatment.
Methods: In study I 10 calves (5 per treatment, body mass about 80 kg, both sexes) were administered 5 or 10 mg DOX in aqeous solution locally in one antebrachiocarpal joint The contralateral joint served as control, injected with saline. Clinical and pathological examination and laboratory analyses of synovial fluid and blood were evaluated.
In study II DOX release kinetics from PLGA microspheres, DOX stability in the microspheres, joint tissue compatibility, and antimicrobial activity of the newly developed DOX containing microspheres was tested *in vitro*.
Results: In study I, the general clinical and pathological outcome between treated and control joints did not differ statistically. Only the metalloproteinase activity in synovial fluid of DOX-treated joints was significantly lower than in control joints; No significant differences were found between the 2 dosage groups.
Study II confirmed a triphasic release profile of DOX-microspheres, an initial burst, a 3 day phase of very little elution and a final almost constant release from day 4 to 15. No signs for preterm degradation were found chromatographically irrespective of γ-sterilization of the PLGA microspheres. Tissue compatibility of the DOX-loaded PLGA microspheres in terms of expression of pro-inflammatory cytokines by cultured synoviocytes was excellent and comparable to control cells. Only nitric oxide production was elevated and close to the positive group stimulated with bacterial polysaccharide. Finally, the antimicrobial activity of DOX released from the microspheres was fully maintained.
Conclusions: DOX may be used intra-articularly and has shown a chondroprotective effect. DOX containing microspheres could provide adequate local drug levels over prolonged periods of time. This should solve the problem of repeated injections.However, *in vivo* and clinical effects remain to be evaluated.

The Chick Embryo as a Tool to Discover and Validate New Therapeutic Targets Against Tumor Angiogenesis

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Background: Recent technical advances such as Affymetrix chicken GeneChips now make the chick embryo a very attractive tool for gene expression studies in physiological angiogenesis, tumor angiogenesis and response to treatment of experimental tumors.
Methods: We have developed a glioma model on the chorioallantoic membrane (CAM) of chick embryos which recapitulates key steps of human tumor progression on a cellular and molecular level. Given the strong parallels between developmental angiogenesis and immature tumor blood vessels, we have created a detailled time-course gene expression atlas which describes for the first time global transcript changes in a developing vascular organ *in vivo*.
Results: Genes which are induced in the vascularization phase of growing tumors on the CAM are also overexpressed in highly malignant patient glioma. When experimental glioma on the CAM were treated with angiogenesis inhibitors, they were significantly smaller than controls, but remaining tumor cells upregulated genes which control invasion and other biological phenomena, which could promote tumor cell survival. Accordingly, high expression levels of two of these genes (PI3 and CHI3L1) were strongly associated with poor survival in a cohort of glioblastoma patients.
We determined human orthologs of hundreds of chicken genes regulated during the maturation of the CAM and assigned *in silico* endothelial cell specificity using novel bioinformatic methods.
Conclusions: 1) The chick embryo allows gene expression studies of physiological and pathological angiogenesis in a defined context, revealing new candidate genes relevant for human disease. 2) The comprehensive molecular map of vascular maturation during developmental angiogenesis constitutes a valuable resource to streamline further research of candidates susceptible to mediate tumor angiogenesis.

Erratum to "Quantification of allantoin in various *Zea mays* L. hybrids by RP-HPLC with UV detection" [Pharmazie, 59(2004) 524-527]

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Background: The *Zea mays* or corn silk is used in the treatment of urinary troubles such as cystitis, urethritis, enuresis, and prostatitis, specifically for acute or chronic inflammation of the urinary system. Allantoin is an astringent and keratolytic. It is frequently used topically as a vulnerary to stimulate tissue repair in suppurating wounds, resistant ulcers, acne, seborrhea, cold sores, psoriasis, hemorrhoid and other anorectal disorders. Accomplishing satisfactory separation of this compound with similar polar constituents in biological samples by high performance liquid chromatography (HPLC) is difficult due to overlapping of peaks. We intended to find a HPLC method for allantoin analysis in Zea mays. A literature study revealed method above which contained a significant error in the identification of the analyte. We duplicated this method and observed that acetone has been mis-identified as allantoin in both the extract and standard solutions. Therefore, aim of this work was to correct this error and present a new analytical method for quantification of allantoin in *Zea mays* by HPLC.
Method: A HPLC validated and improved method for the analysis of allantoin in silk and seed of Zea mays has been developed. Allantoin was extracted from sample and separation of crude extract was achieved on a C_{18} column and phosphate buffer (pH 3.0) as mobile phase at ambient temperature at a flow rate 1.0 mL/min and detected at 210 nm. A comprehensive validation of the method including sensitivity, linearity, repeatability and recovery was conducted.
Results: The calibration curve was linear over the range of 0.2-200 µg/mL with a correlation coefficient (r^2›0.999). Limit of detection (LOD, S/N=3) and limit of quantification (LOQ) values of the allantoin were 0.05 and 0.2 µg/mL respectively. Relative standard deviation (RSD) value of the repeatability was reported within 1.2%. The average recovery of allantoin added to samples was 100.6% with RSD 1.5%. The results showed that the amount of allantoin in samples was between 14 and 271 mg per 100 g of dry plant material.
Conclusion: The present study describes a validated and developed method for quantification of analyte in drug sample and can be used for routine analysis of allantoin as one of the bioactive components in the quality assessment of *Zea mays*.

Studies on new tuberculosis vaccine candidates in animal models

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Background: Tuberculosis (TB) is a major health problem in many countries, especially in low income countries. Globally, TB causes more than 2 million human deaths annually and 1/5 of all adult deaths in developing countries. Most of the world's population is vaccinated with the only available TB vaccine, the Bacillus Calmette-Guérin (BCG) vaccine. The aim of this study is to evaluate the protective efficacy of new TB vaccine candidates.
Methods: New vaccine candidates and adjuvant/delivery systems for mucosal applications were developed and tested in animal models. Lipoarabinomannan (LAM) was purified from a virulent Mtb strain and used to prepare novel oligosaccharide-protein conjugate vaccines. There is a vast number of experimental adjuvants but most of them are intrinsically toxic and only few can be considered for use in man. Aluminum salts are so far the only adjuvants approved for large scale human use. In this study a new adjuvant L3 was investigated. L3 is non-toxic and approved by the Swedish FDA for human phase I/II trials.
Results: The different AM-Prot vaccines and a vaccine based on heat killed whole-cell BCG (H-kBCG) were formulated with L3 and studied for their ability to protect against virulent Mtb challenge in the mouse and guinea pig models. Both types of vaccines, when given nasally, evoked specific and robust cellular and humoral immune responses. Therefore, in this study the role of antibodies in Mtb infection was re-evaluated in passive protection experiments using an AM-specific monoclonal antibodies (MoAb). Mice were infected intravenously with virulent Mtb and the MoAb was added intravenously either prior to or together with the bacteria. The MoAb protected against the infection in terms of a dose-dependent reduction in bacterial load in spleens and lungs, reduced weight loss and, importantly, enhanced long-term survival.
Conclusions: AM-Prot vaccines can, when formulated with the L3 adjuvant and given nasally, provide as good protection as live BCG. The AM-Prot vaccine affords protection both when used as a primary vaccine and as a boost vaccine. H-kBCG vaccine did not protect when administered alone but was protective when given in the L3 adjuvant. The pre-clinical studies of this vaccine candidate are concluded and the vaccine is considered ready for phase I/II trials in man.

The insulin-like growth factor pathway- the key to overcoming resistance in cancer therapy?

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The insulin like growth factor (IGF) pathway is a complex signaling system that has importance in human growth and development. However, dysregulation of this system has been shown to be important in the proliferation and survival in many malignancies including lung, breast and ovarian cancer. Importantly, the IGF signaling has also been implicated as a mechanism of resistance to cytotoxic chemotherapy, hormonal therapy, radiation and biological therapy. Due to the potential of blocking these critical pathways of resistance, novel therapies targeting IGF-1R signaling have been developed and are currently being evaluated clinically. Initial investigations with agents targeting the IGF-1 receptor (IGF-1R) have proven to be very well tolerated and have demonstrated early signs of clinical activity. Among the most common adverse events is hyperglycemia. Recently, demonstration of IGF-1R inhibition having clinical improvement in response to chemotherapy was demonstrated in patients with non-small cell lung cancer. This enhancement of activity was most pronounced in the squamous cell subtype of non-small cell lung cancer, which has a relatively high expression level of IGF-1R. The preclinical and clinical activity of IGF-1R inhibitors will be reviewed. Accumulated data has suggested that crosstalk signaling between the IGF-1R and HER family of receptors (e.g., EGFR, HER2) is responsible for resistance to therapy targeting the individual pathways. Our group has recently demonstrated that the co-inhibition of IGF-1R and HER family of receptors has synergistic activity in multiple models in vitro, through blockade of “crosstalk” signaling. Based on these and other data, we are investigating the clinical activity combined IGF-1R/HER receptor blockade in HER2 positive breast cancer. Through evaluation of phospho-epitopes on circulating tumor cells, we will be investigating whether crosstalk between the IGF-1R and HER2 is clinically apparent and important for resistance to HER2 targeted therapy. These early preclinical and clinical investigations have indicated that inhibition of IGF-1R signaling may be useful in overcoming resistance to many clinically important therapies.

Practice of PK/PD theory with no measuring medicine for new quinolones injectable agents in Japan- As an example of the liver abscess -.

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Background: In the past, liver abscess was associated with high mortality. However, its mortality has recently been reduced due to improvements in diagnostic imaging, progress in drug therapy, and concomitant use of abscess drainage. In the future, shortening of the period of treatment using more effective therapeutic methods, and reduction of patient burden and medical expenses will be of increasing importance. Pazufloxacin(PZFX) and ciprofloxacin (CPFX) are now being marketed in Japan as new quinolone injectable agents. We evaluated three cases of liver abscess being resolved with PZFX. We experienced those of liver abscess in which PZFX was effective, and this case together with a discussion of PK/PD theory is presented with reference to the literature.
Methods: PZFX was intravenously administered to three patients who had undergone abscess drainage at a dose level of 500 mg × 2/day from 2004 to 2005. We aimed Cmax/MIC□10 has been established as an efficacy index for new quinolones at Milan University Hospital in Italy.
We compared PZFX and CPFX using tissue concentration and period of drainage referring to the documents published.
Results: Since the present patient had liver abscess, the maximal concentration in blood was replaced by the maximal concentration in bile for calculations of PZFX concentration. Since the peak of PZFX concentration in bile appears 2-3 hours after administration, the mean concentration in bile (mean ± standard deviation) was calculated using the PZFX concentrations in human bile published in a previous report, and a value of 28.88 ± 16.85μg/mL was obtained. On the other hand, in cases reported previously, the MIC of PZFX against *K. pneumonia* isolated from drainage was <0.5μg/mL, and the finding of concentration in bile/MIC>57.76 was obtained, indicating the usefulness of PK/PD theory.
Conclusions: PZFX therapy thereby allowed the patients to shorten the period of hospital stay. Measurement of blood concentrations in new quinolones during clinical application is not possible at present in Japan. However, we can expect clinical effectiveness with reference to the literature.

The blood-brain barrier and its magic transporters – a pharmacokinetic perspective on how to optimize drug delivery to the brain

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Background: Drug delivery to the brain is intricate due to the tight junctions and active efflux transporters of the blood-brain barrier (BBB). Recently, also active influx has been identified for some drugs. Thus, it would be possible to utilize efflux or influx transporters for the purpose of delivering less or more drug to the brain. Regarding increasing brain drug delivery through active influx, very little is still known. Therefore much more work is needed to understand and map active influx in relation to physico-chemical properties of drug candidates.
Methods: In order to choose the optimal drug candidates for clinically relevant drug delivery to the brain, the methods used in early discovery and development need to be optimised on the relevant measures, i.e. the unbound, pharmacologically active drug concentrations. Unspecific binding in the brain parenchyma distorts the measures when total brain concentrations are utilised. The latter approach has for too long retarded successful development of drugs aiming at the brain. Also, focusing on the permeability (rate) seems to be of less importance for clinical relevance than focusing on the extent of drug delivery to the brain, although the former focus is by far the most common. The advantages with focusing on unbound concentrations include correlations of pharmacologically active concentration at the target site to in vitro receptor binding properties or other pharmacodynamic assays.
Results and Conclusions: We have therefore proposed that brain drug delivery be divided into three main aspects, the rate (PS, CL,in), the extent (Kp,uu) and the affinity of the drug to brain tissue, described by the unbound volume of distribution in the brain (Vu,brain). In this way the unbound concentrations at the target site can be estimated from total brain concentrations and plasma concentrations after measuring fraction unbound, and be related to unbound plasma concentrations. This approach gives quantitative understanding on the role of active efflux or influx in vivo. Rapid methods to study these three aspects are needed in early drug discovery and development. At the same time, in vivo knowledge is needed to validate the methods. We are presently focusing on optimising methods for this purpose.

Rho-Kinase Inhibitors Augment the Inhibitory Effect of Anesthetic Agents on Rat Airway Smooth Muscle Contraction

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Background: Most anesthetic agents relax airway smooth muscle (ASM). ASM contraction is caused by both increasing intracellular Ca2+ ([Ca2+]i) and increasing the force at the same [Ca2+]i (increase in Ca2+ sensitivity). The small G-protein RhoA and Rho-kinase (ROCK) play important roles in regulating Ca2+ sensitivity. In this study, we investigated the effects of selective ROCK inhibitors on ASM contraction and the influence of ROCK inhibitors on anesthetic-induced relaxation in ASM to test the hypothesis; although both anesthetics and ROCK inhibitors relax ASM independently, anesthetic-induced relaxation would be enhanced by addition of a low concentration ROCK inhibitor.
Methods: This study included 50 male Wistar rats (6 weeks, weight 180 - 220g). Ring strips from intrapulmonary bronchus were placed in 400-μL organ baths containing Krebs–Henseleit solution. After obtaining stable contraction with 30 μM acetylcholine (ACh), isometric forces were measured with the following protocols: A) Y-27632 (0.01 – 300 μM), fasudil (0.01 – 100 μM), or H-1152 (0.01 - 100 μM) were cumulatively applied. B) propofol (1 μM – 1 mM), with or without Y-27632, fasudil or H-1152 (0.03, 0.1 μM), was cumulatively applied. C) isoflurane (0.5 – 4.0%), with or without Y-27632 (1 μM), was cumulatively applied. Statistical significance of difference between groups was determined by Two-way analysis of variance (ANOVA), followed by Bonferroni’s test ($p<0.05$ was considered significant).
Results: (A) All ROCK inhibitors, especially H-1152, produced concentration-dependent relaxation.(n=5 each) (B) 0.03 μM Y-27632 and fasudil did not affect the relaxation by propofol, while 0.1 μM both agents significantly shifted concentration-response curves to the left (p=0.040 (Y-27632), p=0.023 (Fasudil)) (n=5 each). H-1152 (0.03 and 0.1 μM) significantly shifted the concentration-response curve to the left ($p<0.001$). (n=5 each) (C) Y-27632 significantly shifted the concentration-response curve for isoflurane to the left. ($P<0.001$) (n=5)
Conclusions: 1) ROCK inhibitors augment anesthetics-induced relaxation of rat ASM. 2) Combined use of ROCK inhibitor and anesthetics may be useful for anesthetic managements and the treatment of asthmatic patients.

Polyethylene Glycol Gold Coated Nanoparticles for the Enhancement of the Efficacy of a Specific Nutrient Synergy

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The effects of nanoparticles on the antiproliferative efficacy of a Specific Nutrient Synergy (SNS) was investigated in both HTLV-1 infected (C91-PL and HuT-102) and non-infected (CEM and Jurkat) malignant T-cells. In all the cell lines tested, the results indicated an enhancement of the efficacy of the SNS in the presence of 0.536 µM polyethylene glycol gold coated nanoparticles using 100 and 200 µg/ml of SNS at 48h and 96h. This effect was molecular weight dependent with maximum effect at 5,000; 10,000 KDa. PEG-coated nanoparticles were more effective than their uncoated counterparts. In addition, the simultaneous or sequential application of SNS and nanoparticles did not affect the overall performance of the SNS. This study suggests a possible role of nanoparticles as a drug delivery vehicle. Future work needs to elucidate the underlying mechanism.

Vaccine Adjuvants for Sexually Transmitted Infections: Recent Developments and Future Challenges

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Background: The vast majority of sexually transmitted infections occur through heterosexual contacts. Globally, rates of sexually transmitted infections among women particularly those in their reproductive age continue to rise disproportionately. The failure to date to produce effective vaccines against sexually transmitted chronic infections including HIV and genital herpes infections, using the empirical approaches successful in the case of diseases such as tetanus and hepatitis B has uncovered a need to develop rational vaccines tailored to elicit specific immune response. Further, since sexually transmitted pathogens invade through and cause disease at the mucosal surfaces, there is a drive to develop vaccines or intervention approaches targeted to the mucosal surfaces.
Most vaccines presently under investigation represent recombinant or highly purified subunit components of pathogens and hence lack most of the features of the original pathogens such as the inherent immunostimulatory property. This calls for the development of safe and potent immunologic adjuvants capable of generating a broad and long-lasting immunity to. Recent advances in immunology have revealed the potential of the stimulators of innate immunity, including Toll-like receptor (TLR) agonists and other non TLR targeting immunostimulators hold promise as vaccine adjuvants. Nonetheless, the potential risk of reactogenicity associated with some of the recently developed immunopotentiators requires special attention.
Results: We were the first to show that CpG oligodeoxynucleotide, a TLR9 agonist, can serve as potent inducer of innate protective immunity in the murine female genital tract, and could also document that CpG can work as a potent vaginal adjuvant. We could also recently document that mucosal vaccination (vaginal and rectal) can elicit protective immunity against genital herpes infection in the female genital tract independent of the use of Toll/MYD88 signaling pathway. This led us to the discovery of a non-TLR targeting immunostimulator, namely alfa-galactosyl ceramide, a synthetic NKT cell ligand, as potent mucosal adjuvant to induce potent protective immunity in the female genital tract.
Conclusion: Recent developments in vaccine adjuvants for generation of protective immunity in the female genital tract, including results from our lab provide a new ground work to develop mucosal adjuvants to mount protective immunity in the female genital tract to counter sexually transmitted infections in humans.

Selective inhibition of signal peptide-dependent cotranslational translocation by the cyclopeptolide CAM741

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Background: The cyclopeptolide CAM741, a derivative of the natural compound Hun-7293, is a potent and selective inhibitor of vascular cell adhesion molecule 1 (VCAM1) expression on endothelial cells.
Results: Extensive studies on the mechanism of action led to the identification of the signal peptide of VCAM1 as target of the compound action. The inhibitory effect of the compound takes place at the signal peptide-dependent process of cotranslational translocation. While CAM741 does not inhibit targeting of the VCAM1 nascent chains to the Sec61 translocon, it inhibits the translocation of the polypeptide chains to the luminal side of the endoplasmic reticulum. Chemical crosslinking further demonstrated that targeted VCAM1 nascent chains are differently associated with the translocon in the presence of compound, and that also the signal peptide itself shows altered positioning within the Sec61 translocon. During a search for other CAM741-sensitive signal peptides, that of vascular endothelial growth factor-A (VEGF) was identified as another target of the compound. Although both signal peptides are sensitive to CAM741, they do not share any obvious similarities within their primary sequence. However, mutagenesis of both signal peptides demonstrated that by increasing the hydrophobicity and decreasing the flexibility of the signal peptides, sensitivity to CAM741 decreased.
Conclusions: Our current model of the compound action depicts that the efficiency of signal peptide binding to the Sec61 translocon at least contributes to the inhibitory action of CAM741 and that the compound competes with the incoming signal peptide for translocon binding. Further investigations on the mechanism of translocation inhibition and the search for other sensitive signal peptides should help to dissect the mode of action and to understand the process of cotranslational translocation driven by signal peptides. These studies provided the first proof-of-principle that the process of cotranslational translocation can be inhibited in a signal peptide-specific manner without affecting the overall translocation process of other polypeptides.

Rationale for the use of memantine in the treatment of glaucoma and other neurodegenerative disease.

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Background Activity of NMDA (N-Methyl-D-Aspartate) type glutamatergic ion channels (excitotoxicity) has been implicated in injury to CNS neurons for a wide range of disorders including glaucoma and retinal disease. Memantine (1-amino-3,5-dimethyladamantane), an NMDA channel blocker, has been approved for the treatment of Alzheimer's disease and has been shown to be effective for reduction of neuronal injury in animal models of CNS disease. In the experiments described here, memantine was tested for its ability to reverse excitotoxicity in an isolated retina preparation and also for its efficacy to reduce injury in animal models of chronic glaucoma.
Methods In-vitro recordings of the ERG (electroretinogram) and spiking activity of retinal ganglion cells (RGCs) were made from rabbit retinas. Excitotoxicity was induced either by application of NMDA, perfusion with zero Mg^{++} medium, or block of glutamate transporters with TBOA (DL-threo-β-Benzyloxyaspartic acid). For in-vivo studies, experimental glaucoma was induced in one eye by laser injury of aqueous drainage vessels, resulting in chronic elevation of intraocular pressure (IOP). Glaucomatous injury was assessed using electrophysiological recordings of the ERG and VEP (visually-evoked cortical potential) in a monkey model and also using histological measures of RGC survival in both rat and monkey models.
Results Experimental excitotoxicity was associated with an increase in tonic RGC spiking activity and a decrease in spike amplitude. Memantine (10 µM) reversed excitotoxicity induced by all three experimental conditions. Applied in isolation, memantine produced dose-dependent delays in ERG and RGC responses. Experimental glaucoma rats treated systemically with memantine (3 mg/kg-day via osmotic pump) lost on average 7% of the RGCs in the glaucomatous eye compared with a 31% loss in vehicle treated animals ($p < 0.001$). Glaucoma monkeys treated orally with 4 mg/kg-day memantine had better preservation of ERG ($p = 0.03$) and VEP ($p = 0.04$) responses compared to vehicle treated animals and also showed better preservation of RGCs in the inferior part of the retina ($p = 0.006$). Memantine had no effect on histological or functional measures in normotensive eyes.
Conclusions Memantine was effective to reduce neuronal injury at concentrations that have little or no effect on normal function.

Proton Transport Inhibitors (PTI) as Selective Anticancer Agents

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CONFERENCE OUTLINE AND SUMMARY

"We can only cure what we can understand first". Otto Warburg

A proton [H+]-related mechanism underlying the initiation and progression of the neoplastic process has been recently described by different research groups. Regardless of their origin and genetic background, all cancer cells and tissues have a pivotal energetic and homeostatic disturbance of their metabolism that is completely different from all normal tissues: an aberrant regulation of hydrogen ion dynamics leading to an intracellular pH to extracellular pH (?pHi to ?pHe), gradient reversal of cancer cells and tissues, that leads to an interstitial acid microenvironment secondary to an initial, specific and etiopathogenic intracellular alkalosis. This specific abnormality is increasingly considered as one of the most sensitive and differential hallmarks of cancer. This approach, which focuses on the relationships among the intracellular and the extracellular dynamics of the hydrogen ion permits the creation of a unifying view of several of the most important fields of cancer research, from etiopathogenesis, cancer cell metabolism and neovascularisation, to multiple drug resistance (MDR), selective apoptosis, the metastatic process, cancer chemotherapy and even the spontaneous regression of cancer (SRC). The integral and rational perspective behind these findings is likely to open new pathways towards the development of more selective and less toxic therapeutic measures for all malignant diseases. New therapeutic approaches are advanced.

Hierarchy of Immune Responses behind the Blood-Brain-Barrier (BBB) in the Normal Brain: Implications for the development of CNS Diseases and Treatment

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Background: Interactions between the brain and immune system are hierarchical and highly regulated. We have shown that antigens microinfused into the rodent brain reach cervical lymph nodes by outflow pathways for brain fluids along cranial nerves and stimulate a specific response in nodes within days. Tumor cells in the brain activate cytotoxic T lymphocytes (CTL) but do not destroy the brain tumor due to suppression of CTL activity by TGF-ß in cerebrospinal fluid. Delayed type hypersensitivity response to microinfused protein fails to develop while the **same dose** elicits a robust serum antibody (Ab) response and intrathecal Ab synthesis, without inflammation, that persists for months. Aim: To determine if the presence of Abs behind the BBB can alter brain function if they cross-react with brain antigens.
Methods: Multiple studies **(I-IV)** were done using a rodent model with normal BBB function. A cannula was stereotaxically implanted into rat brain and 7 days later, dilute sera or immunoglobulins (Igs) with anti-neuronal activity were microinfused (Alzet® pump) through the cannula. Controls received normal sera or Igs. In study **I**, sera or Igs from children with Tourettes Syndrome (TS) were microinfused bilaterally into caudate nucleus (CN) and rats were assessed for stereotypies and Ig binding to neurons. In studies **II-IV,** cannula placement was into the region of the subthalamic nucleus (STN) and the turning response to s.c. injection of apomorphine was measured following unilateral microinfusion of: sera from children with Sydenham's Chorea (SC), **Study II;** rat anti-sera against rheumatogenic streptococci (rStrep), which contains brain-cross-reactive epitopes, **Study III;** and rabbit-anti-Igs against a peptide of oligodendrogliocyte myelin glycoprotein, **Study IV**.
Results: Rats microinfused with TS sera or Igs **(I)** had significant changes in spontaneous behavior (licking and vocalization p< 0.04)) and TS-Igs were bound to neurons in CN. Rats receiving a microinfusion of anti-neuronal Abs into STN **(II-IV)** had significantly greater ipsilateral rotational turning behavior in response to the bioassay compared to control rats. The altered turning response persisted for weeks. In Study **II**, Igs were bound to neurons within the ventral striatum, an area linked to movement.
Conclusion: Antibodies capable of binding brain antigens can affect brain function if they are placed behind the BBB. Affinity of Ehrlich's magic bullets (Ab) and the brain milieu, supportive of Ab synthesis, may be a double-edged sword if synthesized Abs recognize brain epitopes.

The Development of Tumor-Inhibiting Metal Complexes: (Multinuclear) Metal Complexes and Mode-of-Action Studies

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Background: Platinum-based drugs are widely used in the therapy of cancer although being only active in a limited number of tumors and often exhibiting serious side effects. Ruthenium compounds are the best developed metal-based non-platinum anticancer agents, in particular with KP1019 and NAMI-A, two ruthenium(III) compounds, undergoing clinical trials. In recent years, organometallic ruthenium(II)-arene compounds moved into the focus of interest and compounds with activity against primary tumors and antimetastatic activity were reported.
Methods: The synthesis, (bio)analytical characterization and *in vitro* anticancer studies of dinuclear (η^6-*p*-cymene)ruthenium(II) complexes with varying spacer length are reported. The compounds were characterized by NMR spectroscopy and ESI mass spectrometry, and the molecular structure of 1,6-bis{chlorido[3-(oxo-κ*O*)-2-methyl-4-pyridinonato-κ*O*4](η^6-*p*-isopropyltoluene)ruthenium}hexane was determined by X-ray diffraction analysis.
Results: The coupling of two (η^6-*p*-cymene)ruthenium(II) moieties *via* alkyl-pyridinone spacers (alkyl = propane, hexane, dodecane) resulted in compounds **1**–**3** with IC_{50} values in the low micromolar range against the human tumor cell lines A2780 and SW480, whereas the mononuclear analogue **4** is not active. The anticancer activity was found to be dependent of the spacer-length (see Table), which also influences the lipophilicity of the complexes.

Compound	IC_{50} / μM	
	A2780	SW480
1 (C3)	25 ± 2	62 ± 14
2 (C6)	30 ± 6	26 ± 8
3 (C12)	1.5 ± 0.3	0.29 ± 0.05
4	>100	>100

Conclusions: Ru(II)–arene metallodrugs with spacer-length-dependent activity in human tumor cell lines were developed. Notably, not only is an additive effect of the analogous mononuclear complexes observed but a synergistic effect in two cell lines was present – in the SW480 cells being of extraordinary dimension for Ru compounds.

Orally Ingested Lactoferrin and Glycine Display *in vivo* Synergistic Anti-Inflammatory Activity

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Background: There is a growing awareness of the interaction of food constituents with the immune system. The present studies aim to evaluate immunomodulatory effects of two of these nutritional components, i.e. glycine and lactoferrin.
Methods: Mice orally supplemented with glycine, lactoferrin or a combination, were injected intradermally in the ear, with zymosan. Ear swelling (as a measure for inflammation) and the number of TNF-α producing spleen cells were analysed. In a collagen induced arthritis (CIA) model mice were orally supplemented with a combination of glycine and lactoferrin starting after the second collagen booster. Arthritis development was scored and the pro-inflammatory cytokine levels in the serum were detected.
Results: Glycine and lactoferrin were able to decrease the zymosan induced inflammatory response locally (decreased ear swelling) as well as systemically (reduced number of TNF-α producing spleen cells). Glycine effects (20, 50 and 100 mg/mouse/day) were concentration dependent whereas for lactoferrin only the lowest doses (0.1 and 1 mg/mouse/day) inhibited the inflammatory response significantly. Surprisingly higher doses of lactoferrin (5 and 25 mg/mouse/day) failed to influence the inflammatory reaction. A combination of both nutrients (lactoferrin 0.1mg/mouse/day in combination with glycine 20 or 50 mg/mouse/day) inhibited the zymosan induced ear swelling synergistically. In the CIA model the combination of glycine and lactoferrin (lactoferrin 0.1mg/mouse/day with glycine 20 mg/mouse/day) was able to inhibit arthritis development and decrease the level of pro-inflammatory cytokines in the serum.
Conclusions: The present data indicate that the glycine-lactoferrin concept might offer in the near future a powerful nutritional way in modulating chronic inflammatory diseases.

PACAP Signaling: A Promising Drug Target for Neuropsychological Disorders

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Background: Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide with pleiotropic activities including neurotransmission, neural plasticity, and neurotrophy. After we cloned a cDNA for PACAP-specific (PAC_1) receptor belonging to the Class B GPCR and observed its strong expression in the nervous systems in 1993, we have been focusing on harnessing the therapeutic potential of PACAP signaling.
Methods: To this purpose, we generated knockout mouse models for PACAP and PAC_1 receptor by a gene targeting technique and examined their behavioral phenotypes and pharmacological reposponses to various psychotropic drugs. In addition, we performed a case-control association analysis of schizophrenia for PACAP and PACAP receptor genes on a Japanese population comprising 804 cases and 967 control subjects. Intermediate phenotypes that can be linked to the disease were also analysed.
Results: Mice lacking PACAP displayed neuropsychological abnormalities, including altered psychomotor behaviors, sensorimotor gating deficits as determined by prepulse inhibition, hippocampal long-term potentiation and memory deficits, as well as depression-like behavior, most of which were amenable to treatment of the atypical antipsychotic risperidone and intracerebroventricular injection of PACAP. A case-control genetic association study provided evidence that single nucleotide polymorphism (SNP) variants in the PACAP gene and the PAC1 receptor gene were associated with schizophrenia. The overrepresented allele of the PACAP gene in schizophrenia was also associated with poorer memory performance and reduced hippocampal volume.
Conclusions: 1) These convergent data suggest that alterations in PACAP signaling could contribute to the pathogenesis of schizophrenia. 2) As PACAP has pleiotropic actions, e.g. modulation of various signaling systems such as dopamine and glutamate, PACAP signaling pathway can be a target candidate for new therapies.

Role of Endothelial Cells and Treatment with Gentamicin in Septic Shock

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Background: Endothelial cells (EC) play an important role in the pathogenesis of sepsis. It is known that various stimuli such as release of lipopolysaccharide from the bacterial cell wall can trigger the release of large amount of cytokines which in turn can contribute to the microvascular dysfunction and organ injury. The solution form of gentamicin kills the infectious organism in the flowing blood, but is not able to effectively penetrate the cells lining the blood vessels where the bacteria must have been internalized. Aim: To evaluate the uptake of bacteria such as *E.Coli* by EC and compare the effect of gentamicin in the solution versus the microencapsulated form in targetting the bacteria phagocytosed by the EC.
Methods: 1) Human micro vascular EC were grown until they were 80-90% confluent. Labeled *E.Coli* were exposed to these cells and evaluated for total uptake. The internalized bacteria were then targetted using either the solution or the microencapsulated form of gentamicin. 2) This study included 12 rats and the rats were injected with a single dose of gentamicin (45mg/kg) either in the solution form or the microsphere suspension form. 3) Microspheres were labeled using 100µg/mL of fluorescamine solution and injected intraperitoneally, various organs were isolated and evaluated for microsphere distribution using fluorescent microscope.
Results: Treatment with gentamicin microsphere showed 80% inhibition in the growth compared to the solution form which showed only 50% inhibition in the growth and upake of *E.Coli.* Gentamicin solution showed a half life of 175 minutes whereas the microsphere form had a half life of 1910 minutes. Also the extent of absorption for solution group was 5 times lower than the microsphere group. Liver, lung, heart, spleen and kidney showed a significant distribution of micorspheres
Conclusions: 1) Gentamicin microsphere was more effective in targetting the internalized *E.Coli* compared to its traditional solution form. 2) The microsphere form showed a prolonged residence time compared to the solution form. 3) Biodistribution studies demonstrated the engulfment of microspheres by various organs, thus proving it to be an effective carrier of drug in targetting the internalized bacteria.

Cancer stem cells - towards a new generation of antineoplastic drugs

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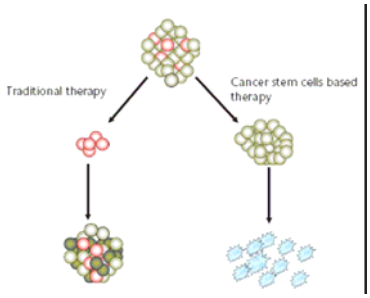
Background: There is evidence accumulating that tumour growth is perpetuated by a rare subpopulation of tumour cells, the cancer stem cells. It is increasingly clear that these cells are causally implicated in therapy resistance, tumour relapse and resulting treatment failure. The typical cancer stem cells are slowly cycling, intrinsically chemoresistant due to a constitutive drug efflux and detoxification activities and display a high level of DNA-repair activity.
Methods: We have performed a literature review of publications directly relating cancer therapy resistance and cancer stem cells. We have developed a method of visualisation of cancer stem cells in living cell cultures, based on isolation of doxorubicin resistant bladder carcinoma cell line and a doxorubicine – responsive GFP – reporter gene construct.
Results and Conclusions: Direct relationship between cancer chemoresistance and cancer stem cells was obtained for a range of tumours, including colon cancer, glioblastoma, hepatocellular carcinoma, endometrial carcinoma, nasopharyngeal carcinoma, ovarian cancer, lung cancer, as well as leukaemia. We are able to visualize naturally chemoresistant cells in a bladder carcinoma cell line and we can show that these intrinsically chemoresistant cells are uniquely clonogenic and responsible for growth restoration after terminating the experimental doxorubicin treatment, thus fulfilling the essential criteria for cancer stem cells. Our method of visualization of cancer stem cells might facilitate identification of therapeutic compound specifically targeting cancer stem cells, which might be an attractive way for future cancer chemotherapy. Targeting normal tissue stem cells by such a stem cell – directed therapy would represent an essential limitation of these approaches of anticancer therapy.



Combination with hyperthermia and radiation contributes to the magic of cisplatin in cancer treatment.

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Background: It has been shown that hyperthermia hyperthermia killas preferentially tumor cells, but not to an extent that it can be used alone. Combination with other treatment modalities is apparent very favourable as many preclinical studies have shown that hyperthermia improves cell killing by radiation and chemotherapy. Promising chemotherapeutic agents in combination with hyperthermia include cisplatin and derivatives, gemcitabine, melphalan, cyclophosphamide, BCNU, bleomycin, vincristine and mitomycin C.
With the clinical application of hyperthermia temperatures in the range of 40 to at maximum 45 °C are used. Sophisticated and safe equipment is commercially available, not only for induction of hyperthermia locally or regionally in the body, but also for advanced planning of treatment. The thermal dose is generally defined as CEM 43 °C, *i.e.* cumulative equivalent minutes at 43 °C and this is used in the treatment planning. Although in many cases temperatures in the range of 42 to 43 °C are prescribed, this goal is often not reached.. Therefore we performed preclinical studies with the aim to study effects at the 'low' temperature of 41 °C in combination with radiation and cisplatin.
DNA double strand breaks (DSBs) induced by ionizing radiation in mammalian cells, can be repaired by homologous recombination (HR) or non-homologous end-joining (NHEJ) in S and G2 phase cells. The results of several studies suggest that the two repair processes can interact. Therefore, inhibition of one of the pathways might result in the shunting of DNA DSB repair to the other pathway. We investigated the effect of temporary HR inhibition by hyperthermia on the repair of ionizing radiation induced DSBs in G2 phase cells. Moreover we studied effects of cisplatin on NHEJ
Methods: The effect of incubation of cells for 1h at 41°C on the accumulation of Rad51, Mre11 and Mdc1 (proteins involved in DNA DSB repair) in ionizing radiation induced foci (IRIF) was studied in two human tumor cell lines. HR efficacy is measured by gene targeting to the Rad54 locus in E14 mouse embryonic stem (ES) cells. The formation of chromosomal fragments (resulting from DNA DSBs) and translocations (resulting from erroneous DNA DSB repair) is measured in G2 phase cells as soon as possible (1h) after irradiation with or without prior incubation of cells at 41°C and/or with cisplatin (an inhibitor of NHEJ).
Results: Incubation of cells at 41°C leads to a temporary inhibition of Rad51 accumulation in IRIF and HR efficacy. This inhibition of HR was accompanied by an increase in the number of chromosomal translocations which could be prevented by incubation of cells with high (≥ 10µM) concentrations of cisplatin. Our results suggest that in G2 phase of the cell cycle the decision which pathway to use for repair of IR-induced DSBs is made early after damage induction. After inhibition of HR, DSB repair might be shifted to the error-prone NHEJ pathway resulting in the rapid formation of chromosomal translocations.
Conclusion: These preclinical results point to favourable effects when three modalities are combined, also at relatively low hyperthermic temperatures. Three modality treatment is currently investigated as an experimental therapy in the clinic.

Gold activates mast cells through L- type calcium channels.

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Background:. Xenobiotic heavy metals including mercury, gold and silver have been shown to highly induce allergy and autoimmunity in genetically susceptible humans and/or experimental animals. Mast cells are implicated to play a role in the development of these adverse immunological reactions. Recently, it is suggested that both allergic and autoimmune mechanisms are involved in autoimmune diseases, including multiple sclerosis, a metal-associated disease. We have previously shown that L-type calcium channels (LTCCs) play a critical role in regulating mast cell activation. Here we demonstrate that gold activates mast cells through LTCCs via the production of H_2O_2.
Methods: Degranulation was determined by β-hexosaminidase and leukotriene C_4 (LTC_4) secretion was measured using an enzyme-linked immunosorbent assay (ELISA). The production of intracellular H_2O_2 was mesuered using DCFH-DA by flow cytometer. The cytosolic calcium concentration ($[Ca^{2+}]_i$) was measured using the Fluo3/AM.
Results: Au(□) at concentrations of raging from 10 μM to 100 μM dose-dependently induced degranulation and LTC_4 secretion with displaying a minimal cytotoxity. In parallel, Au(□) stimulated the production of intracellular H_2O_2 and scavenging the oxidant by the glutathione peroxidase mimetic ebselen blocked $[Ca^{2+}]_i$ increase, degranulation, and LTC_4 secretion. Subsequent studies revealed that Au(□) stimulated LTCC activity, which was activated by H_2O_2. The effects of Au(□) were patially similar to those of Hg(□) and Ag(□). Further investigations on the role for LTCCs using LTCC gene silencing are underway.
Conclusions: Au(□) appears to utilize a unique ROS- and LTCC-dependent mechanism which is partially overlapping with those activated by Hg(□) and Ag(□). This finding may explain the fact that the three xenobiotic heavy metals induce autoimmunity by similar but not identical mechanisms.

Type II Alveolar Epithelial cells play a role in pulmonary host defense system against infectious disease.

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The mechanism of immune defense against pathogens in the lung, has so far been poorly understood. Here, we show that human type II alveolar epithelial cells play a key role in defense via interactions between B7h, also known as ICOS ligand, and its receptor ICOS expressed on activated T cells. The A 549 alveolar type II cell line abundantly expresses B7-H2, CD40 and B7-1, but not B7-2 or hGL50. TNF-α significantly induced B7-H2 and CD40 expression by A549 cells, but had no effect on B7-1 or B7-2 expression. TNF-α deficient mice exhibited low B7-H2 expression on alveolar epithelial cells in comparison with wild type mice. Co-culture of TNF-α pre-stimulated A549 cells with $CD4^+$ T cells promoted CD154 expression, $CD4^+$ T cell proliferation and cytokine production, especially IFN-γ. Monocytes-derived TNF-α in combination with IFN-γ and LPS markedly induced B7-H2 expression in A549 cells. Furthermore, *in vivo* experiments demonstrated TNF-α-inducibility of B7-H2 in alveolar epithelial type II cells. B7-H2 might play a key role in pulmonary host defense system against infectious disease, for instance *Pneumocystis carinii*. This study thus identifies a unique costimulatory pathway via alveolar epithelial type II cells that preferentially affects T-helper cell function, implying that alveolar epithelial type II cells play a crucial role in innate immunity in the lung by regulating IFN-γ-synthesis via B7-H2/ICOS interactions.

Increase of fibrin network porosity and the consequent fibrinolysis as an anticoagulant effect of acetylsalicylic acid

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In patients with stable angina pectoris, the fibrin network permeability shown as the Darcy constant – Ks was significantly enhanced during acetylsalicylic acid (ASA) therapy. Interestingly, a daily intake of 75 mg gave a greater increase in fibrin gel permeability (+92%) than an intake of 320 mg (+5%) in another investigation involving healthy volunteers.
Since above assessments were made in platelet-poor plasma samples, the fibrin formation can be manipulated by numerous pro- and/or anti-coagulation factors in plasma. To confirm whether ASA is able to depress fibrinogen clotting property and thus modify the structure of fibrin network, purified fibrinogen was incubated with different concentrations (0.02 - 2.56 mmol/L) of ASA; a dialysis followed to remove residual drug and its hydrolysis products. The fibrinogen–ASA product was examined with regard to the fibrin network porosity and fibrin resistance to plasmin. Results showed that fibrinogen "clotting time" kept unaffected in all the treated samples. Ks levels were increased and then decreased when ASA levels varied from 0 to 0.08 mmol/L and then to 2.56 mmol/L, respectively. This outcome of fibrin gel permeability was supported by the findings from analysis of 3D-confocal microscopy and from that of fibrin fiber thickness.
Thus, both the studies in vivo and in vitro confirm that fibrinogen clotting property is partially impaired by ASA in a low dose-dependent way. The formed looser network with thicker fibrin fibers favours fibrinolysis, which should be one anticoagulant effect of this therapy.

Cardiotoxicity Plagues Bupivacaine

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Background: Bupivacaine was introduced into clinical practice as a local anesthetic in 1963. Not until 1979 did serious concerns about bupivacaine-induced cardiotoxicity surface following fetal-maternal deaths when bupivacaine was used for epidural analgesia during childbirth. This led to refinement in assumptions about structure-activity relationships for local anesthetics and investigations that furthered understanding of the biological actions of this class of drugs.
Methods: Literature reports of clinical, translational and fundamental investigations of the cardiotoxic effects of bupivacaine were reviewed. Key observations were summarized.
Results: Resuscitation from bupivacaine induced cardiovascular collapse is difficult. The ratio of bupivacaine central nervous system toxic dose to cardiovascular toxic dose is narrower than that for other local anesthetics. Kinetic difference in binding to sodium channel sites influences relative difficulty in resuscitation from bupivacaine cardiotoxicity vs other local anesthetics. The kinetic differences also influence pro and anti arrhythmic activity. Bupivacaine differs from other local anesthetics with respect to the spectrum of effects it has on voltage gated ion channels and on ligand gated receptor signaling. The S isomer of bupivacaine is more potent and less toxic than the R isomer or racemic mixture.
Conclusion: After being considered a "silver bullet" for producing long lasting local or regional anesthesia for 16 years, bupivacaine now is plagued by cardiotoxic effects that sets it apart from other clinically used local anesthetics.

Biopharmaceuticals in plants; toward the next century of medicine.'

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Plants present a novel means by which large quantities of vaccine and therapeutic proteins can be produced in a safe and cost-effective manner. Biopharmaceuticals produced in plants are easy to store, require fewer timely and expensive purification steps, and lack the containment risks associated with proteins produced in animal or bacterial expression systems. Over the past decade, much progress has been made with respect to the development of vaccines, antibodies and other therapeutic proteins. This presentation outlines the steps involved in the generation of transgenic plants, the engineering of plant virus expression vectors for transient expression of vaccine proteins and other therapeutics in plant tissue, and the advantages of this technology over the use of conventional transgenic plants. An investigation into the basis of mucosal immunity using plant-based oral vaccines is addressed. The scale-up of plant-derived vaccine proteins in entire crops or in large batch cell suspension cultures is covered, as is the development of clinical trials utilizing plant-derived biopharmaceutical proteins. Risks involved and biosafety concerns regarding plant-derived biopharmaceuticals are investigated. The presentation will conclude with a discussion of the future of plant-based vaccines and other therapeutic proteins in human and veterinary medicine with respect to commercial viability and as a tool to improve global public health.

EGFRvIII-targeted vaccine (CDX-110) induces immune responses and prolongs survival when given with temozolomide in GBM patients

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Background: Conventional therapies for GBM fail to target tumor cells exclusively. The epidermal growth factor receptor variant III (EGFRvIII) is a consistent and immunogenic mutation that is not expressed in any normal tissues, but is widely expressed in GBMs and other neoplasms making it an attractive target for active immunotherapy.
Methods: A phase II multi-center clinical trial was undertaken to assess the immunogenicity and efficacy of an EGFRvIII-specific peptide vaccine in patients with newly-diagnosed, EGFRvIII+ GBM in combination with simultaneous standard or continuous temozolomide (TMZ). After gross-total resection and radiation/TMZ (75mg/m^2/d), consecutive cohorts received monthly cycles of 200 mg/m^2 (N=13) or continuous 100 mg/m^2 (N=8) TMZ simultaneous with intradermal vaccinations with an EGFRvIII-specific peptide (PEPvIII) conjugated to keyhole limpet hemocyanin (KLH) until tumor progression or death.
Results: 21 patients were enrolled. There were no significant differences in vaccine immunogenicity (*P*>0.999; binomial proportions), PFS (*P*=0.7979; logrank), or OS (*P*=0.7728; logrank) between TMZ regimens. Although TMZ induced Grade II lymphopenia in 53.8% of patients, the co-administration of TMZ with the EGFRvIII vaccine (CDX-110) results in strong sustained immune responses to EGFRvIII in 100% (CI_{95}: 0.72, 1.00) of evaluated patients. Median PFS was 16.6 months (CI_{95}: 9.1, 22.7) and median survival was 33.1 months (CI_{95}: 18.2, infinity). The survival of the vaccinated patients exceeded a matched historical control group (14.3 months; CI_{95}: 13.0, 16.2)(P<0.0001) and a subgroup treated with TMZ (15.2; CI_{95}: 13.9, 20.5)(P=0.0078) and is equivalent to the results seen in patients vaccinated without simultaneous temozolomide (*P*=0.4108).
Conclusion: CDX-110 peptide vaccination with standard of care temozolomide in patients with GBM appears very promising and is under investigation in a phase III, randomized clinical trial.

Matching the Individual Patient to the Results of Large Clinical Trials and Testing the Null Hypothesis Using Fuzzy Theory

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Background: Translation of results of large clinical trials to the individual patient who always is to some degree and in many ways unlike any patient in that trial is imprecise.
Methods: We hypothesized that 1) fuzzy measures of subsethood and entropy precisely match any individual patient to the average patient of any large clinical trial, and 2) that fuzzy subsethood and entropy can be used in the experimental setting to precisely measure the difference between patient states at different points in time and between groups of patients. Fuzzy entropy is the measure of the sameness or non difference between elements and is valued in the unit interval of zero to one. Elements of a fuzzy set are each valued in the unit interval. One patient can be represented by a fuzzy set of elements of biophysiological or other clinical interest. The unit interval value of any element is determined by laboratory or clinical scale measurement followed by normalization or by expert or consensus assignment. In the geometric space of fuzzy theory, the unit hypercube, one fuzzy set as point can represent a patient.
Results: Fuzzy subsethood measures the degree to which fuzzy set A belongs to fuzzy set B. The degree to which fuzzy set A belongs to B and B to A is a measure of the similarity between fuzzy sets A and B. The measure of difference between conditions, unknown or unmeasured variables, of two fuzzy sets A and B is measured by K, derived from fuzzy subsethood, and when accounted for gives a true comparison of fuzzy sets A and B. Following one patient from fuzzy state A to B that patient has changed from one point in time to another. The subsethood measure compares these states. In the experimental setting, this measure is applied to each patient of control and experimental groups. When testing the null hypothesis, the fuzzy entropy measure is the measure of “no difference” between control and experimental patient (s). A fuzzy entropy measure of 1 confirms the null hypothesis. A measure of match between a patient at the bedside and the average patient of any clinical trial is the measure of similarity of those patients accounting for the difference in their context. This measure falls within the unit interval and can be multiplied by the risk reduction, or other statistic of the trial to reach a predictive value for that unique patient.
Conclusion: Fuzzy measures allow statistical results of large clinical trials to be exactly matched to the unique individual patient at the bedside.

Aciclovir- a Nearly Atoxic Antiviral Drug with Severe Neurotoxic Side Effects- a Retrospective Review of 280 Cases and the Importance of Analysing the Aciclovir Metabolite CMMG.

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Background: Acyclovir (ACV) and its prodrug valaciclovir (VACV) is an effective agent against herpes simplex (HSV) and varicella zoster virus (VZV) infections. It is regarded as a nearly non-toxic drug. However, acyclovir-induced neurotoxicity (AIN) has been reported, predominantly in patients with renal impairment. AIN may be difficult to distinguish from the CNS-infection itself. We have earlier shown that the metabolite of aciclovir, 9-carboxymethoxymethylguanine (CMMG), is inconsistently increased and above 10 µmol/L in serum (S) in AIN. This study elucidated if AIN and HSV encephalitis symptoms could be separated from each other and if measurement of CMMG could be a marker of AIN.
Methods: Published case reports on suspected AIN, cases reported to the Swedish adverse drug reactions database “SWEDIS”, and cases investigated at the Karolinska University Hospital were reviewed. Type and frequency of ACV side-effects, renal function at the start of (V)ACV treatment, and serum concentrations of ACV and CMMG were studied. Three published reports on herpes encephalitis and the type and frequency of the initial symptoms were also included.
Results: 280 patients with AIN were found. Sixty-five percent were treated due to VZV, 18% due to HSV and 17% due to other causes. Chronic renal failure was present in 168 (60%) and acute renal failure or increasing S-creatinine in fifty-six patients (20%). The most frequent CNS-symptoms were confusion/disorientation, hallucinations and fatigue. ACV (N=124) and CMMG (N=77) concentrations were (mean ± SD) 44.4 ± 55.0 µmol/L and 38.3 ± 38.6 µmol/L, respectively. Sixty-two of the 77 AIN patients (81%) with S-CMMG had a concentration above 10 µmol/L. Patients with HSV encephalitis (N=197) presented with altered consiousness, fever, personality changes, confusion/disorientation and seizures.
Conclusions:

1. AIN is not uncommon and probably underdiagnosed, especially as the initial symptoms in patients with AIN or HSV (or other encephalitis) are similar and make it difficult to distinguish between the two states.
2. The majority of patients with AIN had acute or chronic renal failure.
3. Measurement of CMMG might be the first effective tool to distinguish between CNS symptoms from herpes encephalitis or AIN. The method is already in use in several Swedish hospitals with promising results.

Glucagon-like Peptide-1: Broadening the Incretin Concept to Involve Gut Motility; a New Target for Treament of IBS

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Background: Glucagon-like peptide-1, GLP-1, is an incretin, that is a peptide hormone released from the gut and enhances insulin release from the pancreatic islets after food intake. In addition to that GLP-1 is also considered to have smooth muscle relaxing properties and causes a profound slowing of gastric emptying rate, making it ideal for the treatment of diabetes type 2. We have previously shown in rat and man that GLP-1 also inhibits small bowel motility. This has led further to evaluate this endogenous peptide for use in irritable bowel syndrome (IBS), where symptoms most likely are due to pressure build-up in the gut lumen. Therapies are needed for IBS as the impact of this disease is considerable in terms of individual suffering and economic cost. By causing relaxation of gastrointestinal smooth muscle, it is thought that the GLP-1 analog, ROSE-010, should be capable of alleviating pressure build-up in the gut, so reducing IBS-associated pain.
Methods: This phase IIA, prospective, randomized, cross-over, double-blind, placebo-controlled, multicenter study enrolled 166 subjects with irritable bowel syndrome. Subjects received single subcutaneous injections at each clinic visit (placebo, 100 µg or 300 µg ROSE-010) within 1 hour of a pain attack. The primary efficacy endpoint was total pain relief response as evaluated by visual analog scales for pain with a reduction of pain of more than 50% in two hours. Secondary endpoints reflected different aspects of IBS pain relief such as meaningful and cumulative pain relief.
Results: ROSE-010 was superior to placebo (response rate 24.2% in total pain relief response compared with 12.0% for placebo [P=0.0053]) with regards to the primary endpoint. ROSE-010 was also more efficacious than placebo as assessed by maximum total pain relief, area under curve for pain relief and summed pain intensity, intensity of pain and summed pain intensity. In addition, the effect of ROSE-010 displayed a clear relationship to food intake. No hypoglycemia or other safety concerns were identified for ROSE-010.
Conclusions: The GLP-1 analog, ROSE-010 (100 µg or 300 µg), was superior to placebo in relieving acute abdominal pain in irritable bowel syndrome. ROSE-010 was safe and well tolerated. These results show that ROSE-010 is a potential treatment for pain in subjects with IBS.

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Which gliclazide (GLC) formulation tablets, immediate (IR) or modified release (MR), are better therapeutic choice?

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Background: IR and MR formulation tablets are available on the market. New formulation tablets (MR) allowing once-daily administration in patients with type 2 diabetes are supposed to improve patients compliance and enhance the long-term maintenance of blood glucose control. Aims: 1) To follow kinetics of GLC dissolution from commercial (CM) and home-made (HM) IR and MR formulation tablets. 2) To perform bioavailability (BA) studies on CM IR and MR formulation tablets in healthy volunteers. 3) To monitor GLC, fasting glucose (FPG), fasting insulin (FI), glycated hemoglobin (HbA1c) plasma levels in patients with type 2 diabetes. 4) To perform pharmacokinetic and pharmacodynamic experiments on GLC from CMIR and HMMR formulation tablets in normoglycaemia and streptozotocine (STZ)-induced hyperglycaemia rats.
Methods: A BP 2001 dissolution test was used for selected GLC formulation tablets. BA investigations included 10 each normal adult volunteers who received single 80 mg formulation tablet either CMIR or CMMR in a crossover design. Their serum samples were quantified for GLC and FPG. 37 type 2 diabetes outpatients were treated with a CMMR GLC formulation tablet once daily at the dose 30-120 mg, and their plasma levels were monitored for GLC, FPG, FI, and HbA1c. Wistar rats aged 7-8 weeks were also used. They were divided into 3 groups each of 8 rats: 3 normoglycaemia groups and 3 STZ-induced hyperglycaemia groups. The group 1 received a methylcellulose solution as a placebo, and the other groups received a suitable single dose of GLC mini-tablets, either CMIR or HMMR. HMMR mini-tablets consisted of GLC 3.0 mg, lactose 7.5 mg, maltodextrin 200 1.5 mg, and Kollidon SR 10.0 mg. TopFit 2.0 software was used for calculation of pharmacokinetic parameters.
Results: CM IR and MR tablets released GLC *in vitro* either very rapidly (approx. 99% at 100 min) or very slowly (67% at 8 h), respectively. The above tablets produced also significantly different serum concentration-time profiles whose mean Cmax values in healthy volunteers were equal either 3.8 or 0.7 mg/L for CM IR and MR tablets, respectively. GLC outpatients' serum concentrations ranged from 0.38 to 9.43 mg/L and their FPG, HbA1c, and FI mean levels were equal 95-368 mg/L, 4.6-12%, and 8.0-25.5 µU/mL, respectively. MR tablets can be administered at lower GLC doses, because 24 h drug steady-state concentration at 24 h is greater (0.6±0.1 mg/L) if compared to 80 mg CMFR GLC tablets. Any case of hypoglycaemia was not observed in the above patients, because their FPG was never lower than 75 mg/dL. 7 patients produced less FI than required (5.6 µU/mL). It can be also distinguished a group of high FI level (≥15.6 µU/mL) patients who received higher GLC doses. GLC Cmax serum levels after administration CMIR mini-tablets were as it follows: 31.60±9.11 and 38.84±11.48 mg/L for healthy and diabetic rats, respectively. These values were over 8-fold greater than for HMMR mini-tablets. However, mean residence time (MRT) of HMMR tablets was approximately 2 h longer if compared to CMIR tablets. A greater decrease in FPG was observed for healthy rats (47%) as a result perhaps of diminished number of islet cells in STZ-induced hyperglycaemia rats.
Conclusions: 1) The dissolution process of CMIR GLC formulation tablets follows the first-order kinetics, and for MR tablets it proceeds according to a zero-order equation. 2) MR GLC resides in the human body longer at a lower level. 3) It is efficient with respect to HbA1c and FPG levels and safe to avoid hypoglycaemia. 4) Pharmacokinetic profiles of GLC are also remarkably different for IR and MR its formulation tablets in healthy and diabetic rats. 5) Pharmacokinetic profiles of CMMR and HMMR mini-tablets are comparable. 6) MR GLC formulation tablets are of benefit if a mild and an extended effect is desired.

Pharmacokinetic interactions of drugs and fruit juices with carbamazepine in Rat

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Background: Carbamazepine is an antiepireptic agent metabolized by CYP3A; hence CYP3A inhibitors or substrates may interact with carbamazepine. There are, however, only limited data on how to predict drug interactions in humans. Therefore, in this study, we investigated the CYP3A-mediated drug interaction in rat.
Methods: Male Wistar rats, weighing 280 to 300 g, were used in the study. The effects of simvastatin, pomegranate juice, and star fruit juice on carbamazepine pharmacokinetics in rats were evaluated. Simvastatin (10 mg/kg) or 2 ml of juice or water was orally administered to rats (N = 6). Carbamazepine at a dose of 50 mg/kg was orally administered through gastric intubation at 1 h after the pretreatment. Blood samples (approximately 0.2 ml) were collected through the carotid artery at 15 and 30 min and 1, 2, 4, 6, 8, 10, 12, and 24 h after the oral administration of carbamazepine. Analysis of carbamazepine and carbamazepine 10,11-epoxide was performed by high perfomrance liquid chromatography.
Results: In comparison with water, the area under the concentration-time curve (AUC) of carbamazepine was approximately 1.5-fold higher when pomegranate juice was administrated. On the other hand, the elimination half-life of carbamazepine and the AUC ratio of carbamazepine 10,11-epoxide to carbamazepine were not altered by the injection of pomegranate juice. Carbamazepine AUC were increased approximately 1.3-fold when simvastatin or starfruit juice administration. Carbamazepine pharmacokinetic parameters are shown in the table (average ± SEM, *: p<0.05).

parameter	*C*max µmol/ml	*T*max h	AUC µmol*h/ml	*t*1/2 h
Control	53.6 ± 4.5	0.38 ± 0.06	394.0 ± 40.1	9.1 ± 0.6
Pomegranate	74.5 ± 6.6*	0.50 ± 0.11	572.6 ± 32.5*	8.8 ± 0.5

Conclusions: Pomegranate juice, starfruit juice and simvastatin influenced the pharmacokinetics of carbamazepine in rat. These treatment did not affect the $t_{1/2}$ values of carbamazepine and metabolite formation in the systemic circulation. These results suggest that the interacitons would be caused by enteric CYP3A inhibition.

Cost Implications of Oral Treatment of Colorectal Cancer in Germany

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Background: Fluoropyrimidine-based therapies are standard in the treatment of metastatic colorectal cancer. Intravenously administered 5-fluorouracil was the only option before Capecitabine, an oral fluoropyrimidine, became available. As the drug acquisition costs of Capecitabine are considerably higher than those of 5-fluorouracil it was the objective of this study evaluate cost implications of oral chemotherapy with Capecitabine vs. standard fluoropyrimidine-therapies (Mayo Clinic and AIO/Ardalan-regimen), in different treatment settings in Germany.
Methods*:* Costs of fluoropyrimidine-therapies were evaluated for the office-based setting. Physician's fees (89 quarterly fee-listings, 26 patients, 6 office-based oncologists), drug and pharmacy costs and costs for venous port systems and single-use pumps were included. Capecitabine treatment costs were assumed to be identical to the cost of the Mayo Clinic-regimen, except drug administration and acquisition. Based on the frequency of administration of active drugs by office-based oncologists costs were modelled for the hospital sector, i.e. day-case and inpatient treatment. A third-party payer perspective was adopted. Market research data on frequency and setting of use of the evaluated regimens were used to estimate potential overall cost implications.
Results: Treatment costs for a 6-months course in the office-based setting was most expensive with the AIO/Ardalan regimen (€ 18'600) and cheapest with Capecitabine (€ 3'870). Treatment costs in the hospital setting ranged from € 7'070 (Mayo) to € 22'790 (Ardalan, inpatient treatment).
Conclusions: Higher drug acquisition costs of Capecitabine compared to 5-fluorouracil are more than compensated by lower costs for drug administration, resulting in net cost savings for Capecitabine. The most expensive treatment options were the AIO/Ardalan-protocol in both, the office-based and the hospital setting. Capecitabine emerged as the cheapest option in the office-based setting (NA for hospital due to oral administration). Transferring patients to oral capecitabine is likely to result in substantial cost savings. Savings are likely to be even higher if combination therapies with irinotecan or oxaliplatin are considered.

A new focus on atherosclerosis treatment: transialidase from *Trypanosoma cruzi* as an anti-proliferative drug.

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Background – Chagas disease is caused by Trypanosoma cruzi that produces transialidases (TS) and Chagasic patients usually do not have atherosclerosis. Atherosclerosis is an inflammatory multifactorial disease, presenting increased levels of sialic acids (SAs), transferrin and iron in the plaques possibly related with free radical release and inflammation. TS produced by TC may remove SAs from the plaques.
Aim: To report a new way for treating atherosclerosis focusing on multitargets simultaneously: removal SAs with a recombinant TC TS, a metal chelant (PDTC) and antioxidative nanoparticles derived from three plant extracts in an animal model.
Methods - We compared six groups (n=5) of rabbits. GI – normal diet; GII – 1% cholesterol diet for 12 weeks; GIII – 1% cholesterol diet for 12 weeks and, in the last 4 weeks, injection of transialidase (TS) plus PDTC. Groups IV, V and VI received the same scheme of GIII plus aged extracts: Allium sativum (AL); AL+ Ginko biloba (GB) and AL+GB + Zingiber officinale (ZO), respectively. The thoracic aorta atheromas stained with Sudan IV were macroscopically detected and plaque and fat areas in cross sections were microscopically detected using an image analysis system. The LDL in the serum was also measured.
Results – The combined therapy using TS+PDTC and 3 plant extracts was the most effective scheme in reducing atheromas and LDL serum levels to normal values. (Figure and Table below).
Conclusion – The successful treatment of experimental animal atherosclerosis using *T.cruzi* transialidase, PDTC and plant extract nanoparticles combined formulation brings a new promissory way for the treatment of human atherosclerosis.

	Regular diet	1% Cholesterol-enriched-diet for 12 weeks				
Analysis	**Intervention**		TS + PDTC in the last 4 wks			
Mean ± SD	**GI** (control)	**GII** (ch diet)	**GIII** (ch diet + TS + PDTC)	**GIV:** (similar to GIII + AL)	**GV:** (similar to GIV + AL+GB)	**GVI:** (similar to GV + AL GB ZO)
% Total Area Plaque	0 ± 0	75 ± 9	50 ± 3	67 ± 14	42 ± 8	11 ± 1
% Plaque Fat Area	0 ± 0	89 ± 5	50 ± 3	61 ± 10	40 ± 14	17 ± 10
LDL mg/dl	33 ± 24	775 ± 227	743 ± 92	635 ± 60	335 ± 29	18 ± 6

Consequences to morphology of primary neurons as an effect of Nitric Oxide on plasma membrane fluidity

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Nitric Oxide is described as a signal molecule of special importance in the nervous system mainly acting via cyclic guanosine monophosphate which is generated by the Nitric Oxide sensitive guanylate cyclase. However, our group [Grote Westrick, Hippe et al. (in preparation)] could show that Nitric Oxide has a direct effect on the plasma membrane of living cells by demonstrating the same effect on artificial generated liposomes. In consequence of Nitric Oxide donor application membrane fluidity is increased.
Initial experiments have shown that the same effect occurs on neuronal cells. By the use of NOC-18 (Nitric Oxide donor) and L-NAME (Nitric Oxide synthase inhibitor) we show expected morphological changes on primary hippocampal neurons with scanning electron microscopy and immunocytochemical staining in addition to enhanced membrane fluidity measured by fluorescence recovery after photobleaching (FRAP). Further we plan to establish solid state ^{31}P-NMR anisotropy measurements of artificial generated liposomes followed by electron microscopy analysis to detect changes in the membrane of those vesicles. In combination with mass spectrometry measurements we intend to demonstrate the molecular basis of the characterized direct NO effect on neuronal plasma membranes.

Using Immune Complexes to Enhance Antigenicity and Immunogenicity of a Neutralizing Epitope on HIV-1 Envelope gp120

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Background: HIV envelope gp120 is a crucial antigenic target for Ab responses against the virus; but most, if not all, of the critical gp120 epitopes are not readily accessible for Ab recognition or are poorly immunogenic. Since antibodies are known for their capacity to enhance immune responses to specific antigens, we investigated the use of immune complexes as immunogens to improve the overall Ab titers against gp120 as well as to target the Ab response toward specific neutralizing epitopes.
Methods: Immune complexes were made of gp120 from different HIV-1 subtype B strains and human monoclonal Abs (mAbs) to the CD4-binding site (CD4bs) of gp120. The anti-CD4bs mAbs were selected because a) these mAbs can induce significant structural changes in gp120 that potentially expose neutralizing epitopes, and b) the gp120/anti-CD4bs complexes are relatively resistant to degradative enzymes and may serve as a durable antigen source for stimulating B cells and Ab production. The antigenicity of the gp120/anti-CD4bs complexes was examined by ELISA using biotinylated mAbs to different gp120 regions, while the immunogenicity of the complexes was evaluated by immunizing BALB/c mice intraperitoneally with the complexes.
Results: HIV-1 gp120 complexed with anti-CD4bs mAbs displayed higher reactivities with mAbs specific for the neutralizing epitopes in the V3 loop. The enhanced reactivities were observed with gp120 from different HIV-1 strains. Immunization of mice with the complexes also elicited >1 log higher titers of gp120-specific serum IgG and IgA than immunization with uncomplexed gp120 or other gp120/mAb complexes. Notably, the enhanced Ab production was directed against V3, and the use of $gp120_{JRFL}$ bearing V3 of the HIV-1 subtype B consensus sequence increased the cross-reactivity of Abs generated. Importantly, potent neutralizing activity was observed in sera from mice immunized with the gp120/anti-CD4bs complexes, although assessment of the breath of neutralizing activities against a panel of subtype B HIV-1 isolates is still on-going.
Conclusions: Overall, these results indicate that the use of immune complexes is a promising approach to improving the immunogenicity of HIV-1 envelope and to directing the Ab responses toward virus-neutralizing epitopes on this antigen.

Changes in Plasma Protein Binding of an Extensively Bound and Highly Extracted Drug, Propofol, Have Clinical Relevance

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Background: Changes in plasma protein binding have little clinical relevance for most drugs. However, this situation is likely to be clinically important for a limited number of highly cleared drugs that are extensively protein bound, administered intravenously and have a narrow therapeutic index. Propofol has been widely used in the clinical field of anesthesia and intensive care medicine and is one such drug. Thus, changes in plasma protein binding of propofol would be expected to alter disposition of propofol and its potency. Therefore, we have investigated propofol's pharmacokinetics during cardiopulmonary bypass (CPB) to determine whether the predicted theoretical changes actually occur.
Methods: After induction of anesthesia propofol was infused continuously during surgery. Propofol's concentration was measured by HPLC in blood samples collected from the radial artery during surgery at predetermined intervals. The drug's unbound fraction in arterial plasma was estimated via equilibrium dialysis. Bispectral index (BIS) and burst suppression ratio (BSR) were measured continuously to quantify the potency of propofol.
Results: The total concentration of propofol in blood was unchanged during surgery. By contrast, the fraction of unbound propofol in blood increased by 2-fold during cardiopulmonary bypass. BIS was significantly decreased and BSR was significantly increased during CPB.
Conclusions: The potency of propofol significantly increased during CPB without any alteration in the total drug concentration. The enhanced efficacy would be caused by a reduction in plasma binding of the drug. Furthermore, total drug levels of propofol are influenced by cardiac output. We also report the cases of accidental hemorrhagic shock in patients undergoing liver transplantation, where 2-fold increases in both total propofol concentrations and the unbound fraction result in 4-fold increases in the unbound concentrations. Changes in plasma protein binding of propofol would be clinically important.

Rational Design of Specific Inhibitors of γ-Glutamyl Transpeptidase (GGT) and γ-Glutamylcysteine Synthetase (GCS) for Modulating Cellular Glutathione and Redox Status

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Background: Glutathione (γ-Glu-Cys-Gly, GSH) is a ubiquitous tripeptide that serves as a major cellular antioxidant or detoxifying agent. Therefore cellular GSH level is deeply associated with drug resistance of cancer cells and pathogens. GSH biosynthesis is dependent on the activities of γ-glutamylcysteine synthetase (GCS), a second biosynthetic enzyme, and of γ-glutamyl transpeptidase (GGT) that hydrolyzes the extracellular GSH to supply cells with its constituent amino acids. Aims: 1) To develop rationally specific inhibitors of GGT and GCS. 2) To understand the interaction of the inhibitors with the enzymes at the molecular level.
Methods: A series of electrophilic γ-phosphonic diester analogs of GSH were synthesized as mechanism-based inhibitors of GGT. Each inhibitor was evaluated by the second-order rate constant for enzyme inactivation. The structure-activity relationships were used for the active-site mapping. For GCS inhibitors, sulfoximine-based transition-state analogs were synthesized according to the X-ray crystal structure of *E. coli* GCS. The recognition of Cys by an Arg residue of *E. coli* GCS was utilized for the inhibitor design, and a cyano group was introduced as a SH mimic.
Results: The γ-phosphonic diesters served as potent irreversible inhibitors of both *E. coli* and human GGTs by attaching covalently with the catalytic Thr. The potency was highly dependent on the structure mimicking the Cys-Gly moiety, but human GGT was far more selective, suggesting that human GGT served as a "glutathionase" *in vivo*. The γ-phosphonic diesters did not inhibit glutamine amidotransferases. The most potent GGT inhibitor exhibited ca. 6000 times as potent as acivicin, a hitherto used non-selective GGT inhibitor. The introduction of CN group significantly increased the potency of inhibitor for both *E. coli* and a pathogenic Streptococci GCS. The best inhibitor was ca. 2500 times more potent than BSO, a commonly used GCS inhibitor.
Conclusion: The reaction mechanisms and the structure of GSH biosynthetic and degrading enzymes were highly useful for rational design of selective and potent inhibitors. This is the first step toward controlling the cellular GSH levels that is highly promising for combating drug resistance.

Effectiveness and complications of treatment for childhood liver tumors – from the experience of JPLT (Japanese Study Group for Pediatric Liver Tumor) study

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Background: Hepatoblastoma (HB), which is derived from hepatic precursor cells, is a primary liver cancer that occur predominantly in children. The prognosis of children with HB has been improved significantly during the past two decades by the induction of neoadjuvant and adjuvant chemotherapy. JPLT group launched in 1991 is running cooperative treatment studies based on the cisplatin/pirarubicin regimen (CITA) as the first line on HB. Aims: 1) To evaluate the efficacy of JPLT protocol. 2) To evaluate the late complications of the patients by JPLT regimen
Methods: Until 2007, 235 HB cases have been registered in JPLT-2. According to PRETEXT classification, standard risk (SR) tumors (PRETEXT 1-3) without metastasis were 146 and high risk (HR) patients (PRETEXT-4 or metastatic cases) were 89. In JPLT-2 protocol, high dose chemotherapy with stem cell transplantation (SCT) was carried out for metastatic tumors and living-donor liver transplantion (LD-LT) for some PRETEXT-4 tumors. Late complications were evaluated in 126 cases that received complete regimen and survived more than 2 years.
Results: The 3-year overall survival (OS) and event-free survival were 80% (95% in SR and 54% in HR) and 67%, respectively. The response rates of CITA regimen were 82% and 52% and the complete resectability was 87% and 41% in SR and HR cases, respectively. In 23 cases who underwent high dose regimen with SCT, only 13 cases (57%) were cured. In 31 cases who underwent LD-LT, 3-year OS was 76%. Late complications are shown in Table.

Long term toxicity	Grade 1-2	Grade 3-4	Grade 5	total
Growth & development	3	1	-	4
Cardiac	11	2		13
Auditory	17	5		22
Secondary malignancies	-	2	2	4

Conclusions: 1) SR cases had a fair to excellent outcome but, late complications are now problematic. 2) The outcome of HR cases remained poor. Reduction of chemotherapy for SR cases and more promising strategies for HR cases including LT and new targeting drugs should be developed in international collaboration because the case numbers were limited.

Genes commonly upregulated in immortal cancer cells but not in immortalized non-cancerous cells are novel molecular targets for universal anti-cancer strategy

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Background: Molecular targetting anticancer drugs are quite promising in some specific cancers but usually limited to selected patients. It is widely accepted that activation of telomerase induces indefinite proliferation capacity in almost all kind of cancer cells resulting in poor progonosis of patients, but never in normal human cells *in vivo*, resulting in senescence.
Methods: To elucidate the genes responsible for telomerase-mediated cellular immortalization in cancer cells, we established telomerase overexpressing *in vitro* immortalized normal and transformed cells lines by gene transfection of *TERT* and/or SV40 EA. By comparing the expression profiles of them and various cancer-derived cell lines with those of normal cells using microarrays, we explored the genes that are differentially expressed in immortal cancer cells but not in immortalized non-cancerous cells. The characteristics of the candidate genes were examined *in vitro* and in clinical cancer tissues of various organs.
Results: While 3 genes were detected as commonly down-regulated, 34 genes were commonly up-regulated in the cancer cell lines of any origin, except for breast cancer ("*" in Table). Large part of these genes were also upregulated in various cancer tissues but not in *in vitro* immortalized normal cells. Some of them also correlated with advanced stage and/or poor progonosis of the patients. Knockdown by siRNA of these genes demonstrated inhibititory effects on cellular growth in cancer cell lines.
Table. Number of the genes differentially expressed in immortal cancer cells.

Cancer cell line	Lung	Esophagus	Colon etc	Breast	Ovarian	Pancreas	Common
Upregulated	570	196	958	196	935	2553	34*
Downregulated	675	396	747	447	784	1504	3

Conclusions: We have found the candidate genes that may be involeved in cellular immortalization commonly and specifically in cancer cells, but not in normal cells, cooperatively with telomerase. The genes we found are expected to become the novel molecular targets of universally effective anti-cancer therapy.

System approach to Magic Bullets: tissue and cell targeted delivery of HIV and cancer drugs

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The estimated number of drug targets between 1940-1990 was about 500. In post-genomics era, over 1700 human genes express function proteins suitable for serving as drug targets. Despite the drug-target growth, approval of novel therapeutics for human diseases has not increased substantially. In addition to well-documented preclinical challenges (in drug absorption, distribution, metabolism and elimination) in the development of new drug candidates, increasing frequency in drug failures during the late-stage clinical trials are often attributed to toxicity or lack-of-efficacy. Consequently, introduction of new drugs into market is low despite increased expenditure by pharmaceutical industry and government sponsors. To improve success rate, distribution profile of drug targets in "normal" and "cancerous or virus-infected" cell are being elucidated and mapped with the help of various *in vivo*-imaging techniques as a part of increased collaborations among basic, preclinical and clinical scientists, as well as integration of translation research early in drug discovery and development. Even with improved understanding in "biodistribution of drug targets," oral or systemically administered drug molecules must cross a number of physiologic—tissue, cell and enzyme (such as cytochrome P450's metabolism in the gut and liver) barriers before reaching the virus or cancerous cells found in target tissues and cells. A significant fraction of drugs are either eliminated in the gut and liver without ever reaching systemic blood circulation or being metabolized and inactivated. Some of the metabolites also induce untoward responses in liver kidney and other tissues. As a result, current oral anti-HIV drug combination therapies could reduce virus load to undetectable levels in the blood, but could not clear the virus in the tissues such as those in lymphoid tissues and cells. Building on the physiologic and biologic understanding at systemic levels, and applying the advances in drug delivery technologies, we have made progress in improving drug localization at three progressive levels; (1) lymphoid tissues, (2) HIV host cells, (3) HIV drug targets—protease and reverse transcriptase found in HIV infected cells. This presentation will highlight our results in developing a systematic, practical, and novel approach to accomplish these goals using a HIV infected macaques model.

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Pharmacokinetic/pharmacodynamic considerations for inhaled glucocorticoids

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Background: For the last 30 years, pulmonary drug delivery has been successfully employed for topical therapy of pulmonary diseases, with the goal of achieving pronounced pulmonary effect while reducing systemic side effects. The degree of pulmonary targeting is determined by a number of pharmacokinetic (PK) and pharmacodynamic (PD) factors. This presentation will discuss these relationships, and will review the pharmacokinetic and pharmacodynamic properties an inhaled glucocorticoid should provide.
Methods: This presentation is based on previously published in-vitro experiments, PK/PD simulations, animal and clinical studies of glucocorticoid action including the effects of biopharmaceutical parameters on pulmonary selectivity.
Results: Pharmacokinetic/dynamic simulations suggested that pharmacodynamic properties of an inhaled glucocorticoid beneficial for pulmonary targeting are low oral bioavailability, pronounced systemic clearance and distinct pulmonary residence time, while factors such as protein binding and degree of receptor affinity can be adjusted for by dose. PK/PD tools were also suitable to address the question when once-daily glucocorticoids should be administered. A pulmonary targeting model in rats was able to demonstrate the relevance of the results obtained in PK/PD based simulations and revealed the importance of biopharmaceutical optimization for the degree of pulmonary selectivity.

Conclusions: The presented work suggests that the use of PK/PD tools within the rationale drug design is also beneficial when drugs for topical use, such as inhalation, are to be designed.

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Pharmacokinetic-Pharmacodynamic Modeling of Calcium Channel Blockers in Animal Models of Hypertension.

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Background: Pharmacokinetic-pharmacodynamic (PK-PD) properties of calcium channel blockers have been scarcely studied in animal models of hypertension. Aims: 1) To compare different pharmacodynamic models for PK-PD modeling of diltiazem (DTZ). 2) To evaluate pharmacokinetic properties of DTZ in two different models of hypertension, such as aortic coarctated rats (ACo) and spontaneously hypertensive rats (SHR) 2) To establish sensitivity to calcium channel blockade in SHR and ACo rats.
Methods: A "shunt" microdialysis probe was inserted in carotid artery of anaesthetized SHR, ACo and normotensive control rats for determination of DTZ plasma levels and their effects on blood pressure and heart rate after drug administration (1, 3 or 6 $mg.kg^{-1}$, iv). Correlation between DTZ plasma levels and their cardiovascular effects was established by fitting the data to a conventional and modified Emax model.
Results: Volume of distribution and clearance of DTZ was greater in both SHR and ACo with regards to normotensive animals. A good correlation between plasma levels of DTZ and their hypotensive and chronotropic effects was found in all experimental groups using both PK–PD models. Application of the modified Emax model for PK–PD modeling of DTZ allowed a more accurate and precise estimation of PK–PD parameters than the Emax equation do. Initial sensitivity (S_0), estimated by the modified Emax model, to DTZ chronotropic effect was greater in SHR with regards to control animals after administration of 1 mg kg^{-1}. Conversely, no differences were observed in S_0 to DTZ chronotropic effect comparing ACo and normotensive animals. Both SHR and ACo rats showed increased S_0 to DTZ hypotensive effect with regards to normotensive animals.
Conclusions: 1) Modified Emax model allowed both a precise and accurate estimation of PK-PD parameters, suggesting that this pharmacodynamic model is the most suitable for PK-PD modeling of DTZ. 2) ACo and SHR induced profound changes in DTZ pharmacokinetic behaviour, affecting both drug distribution and clearance. 3) Experimental hypertensive rats showed an increased sensitivity to DTZ blood pressure lowering effect.

T-cells as Magic Bullets: Recombinant Vaccine Strategies for Cancer Immunotherapy

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Cancer vaccines can induce T-cell responses that can preferentially target tumor cells while sparing normal cells. Preclinical and clinical investigations currently underway are employing novel strategies for combining vaccines with conventional and experimental anticancer therapies. To date, the FDA has not approved a therapeutic cancer vaccine. However, the results of recent investigations suggest an increasing role for vaccines in new models of combination therapy for many types of cancer. In this talk I will discuss therapeutic cancer strategies that employ vaccines in combination with local radiation, chemotherapy, hormone therapy, and anti-CTLA-4 mAb. Preclinical studies have shown that certain anticancer agents have immune modulatory effects that result in up-regulation of surface expression of MHC molecules, tumor-associated antigens, or Fas on malignant cells, rendering them more susceptible to immune destruction. Preliminary results of clinical studies using combination strategies have demonstrated a postvaccination antigen cascade, prolonged time to disease progression, and improved overall survival. Several larger randomized trials are ongoing, and more are required to support these findings.

The Utility of the Cholestanol:Cholesterol Ratio in Predicting LDL-Cholesterol response to Atorvastatin 80 mg: A paradigm for individualized lipid-lowering therapy

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Background: Cholestanol is an endogenously produced marker of cholesterol absorption that has been validated with sterol balance methodology. A retrospective analysis by the 4S investigators suggested that patients in the highest quartile of the cholestanol:cholesterol ratio (CCR), indicating a cholesterol 'absorber' phenotype did not benefit from statin therapy in the 4S trial. Patients with high CCR have high cholesterol absorption and low synthesis; hence statins are expected to be relatively ineffective in such patients. Since recent meta-analyses have shown that benefit from statin therapy is directly related to LDL-cholesterol (LDL-C) response, we sought to prospectively assess whether the CCR can predict LDL-C response to Atorvastatin and identify statin hypo-responders, defined as an LDL-C response <40%.
Aims: To assess the predictive value of CCR in predicting LDL-C response to Atorvastatin 80mg.
Hypotheses: CCR correlates with the LDL-C response to Atorvastatin. Patients who have a higher CCR will have a suboptimal LDL-C response to statin and the CCR can be used to identify statin hyporesponders (LDL-C response <40%).
Methods: ~60 patients with coronary artery disease or coronary risk-equivalents were recruited into the study. Baseline measures included CCR, baseline lipid panel, insulin, CRP, assessment of metabolic syndrome criteria, blood sampling for genetic analysis and an MRI substudy for patients without contraindications. Patients were given a 6 week course of Atorvastatin 80mg before follow up to determine the lipid and CRP response to the course of therapy.
Major Results: The 6 week course of Atorvastatin reduced LDL-C by a mean of 55.7% ($P<0.01$ for post vs pre-treatment LDL-C values); however, there was marked heterogeneity in the LDL-C response (range 12-80%). Of the baseline variables assessed, only the CCR correlated with the percent LDL-C reduction ($p<0.01$, $R2=0.21$). When the population was divided into quartiles of CCR, statin hypo-responders (LDL-C response <40%) clustered in the patients with the highest CCR (quartile 4 vs quartile 1 $p<0.03$). Patients in the highest quartile (quartile 4) of CCR showed less features of the obesity and the metabolic syndrome than those in the lowest quartile (quartile 1) with significant differences being found in number of metabolic syndrome criteria, HDL-C, VLDL-C, TG, BMI (all $p<0.01$). ROC analysis suggested that the CCR can identify statin hypo-responders (LDL-C response <40%) with a 100% sensitivity and 62% specificity (Area under curve = 0.85, $p<0.01$).
Significance: This is a highly significant finding and highly applicable to personalized medicine in the area of lipid-lowering therapy. Of note, pharmacogenomic approaches to variability in statin response typically yield R2s of 0.01-0.02. We propose that the CCR can be used to predict LDL-C response to statin therapy and identify statin hypo-responders who might be expeditiously treated with anti-resorptive co-therapy such as ezetimibe. These findings are particularly applicable to high risk cardiovascular patients where aggressive lipid-lowering therapy is warranted.

A Genomic Parasite in the Evolution of Metazoan Development Evolvability and Evolutionary Constraints as Fingerprints of Selection

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It is a challenge to understand how development emerged as a mechanism to dismantle and dismiss the intromission of foreign parasites in order to consolidate a higher-level multicellular unit of selection where more heritable variations in fitness, required for complex organization, can be procured.levels in the biological hierarchy-genes,networks of genes, chromosomes, cells, organisms etc.,possess heritable variations in fitness to varying degrees,and as such they function as units of selection in the evolutionary process.to pass from each of these levels to the next constitutes a major transition in evolutionary history.when analyzing the splendid road epitomized by these transitions in the units of selection, it is only possible to conceive of three processes:1)the molecular recognition of the convenience of exchanging the higher energy cost of cooperating cells with more fitness than single cells selection. After that first recognition the emergence of cooperation among cells is possible.2)the establishment of the mechanisms to regulate conflict, and 3)the regulation of cell differentiation and compartmentalization.

Non-Selective and Selective Adenosine Receptor Agonists in the Treatment of Radiation- and Chemotherapy-Induced Myelosuppression

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Background: Adenosine acts as a regulator of many cellular functions including those of cell proliferation and differentiation. Regulatory role of extracellular adenosine is based on the activation of specific cell surface receptors. Adenosine receptors can be classified as A_1, A_{2A}, A_{2B}, and A_3. We have investigated the role of adenosine receptor agonists in regulation of processes proceeding in hematopoiesis suppressed by ionizing radiation or cytotoxic drugs.
Methods: The studies have been performed on mice. The animals were exposed to ionizing radiation or to cytotoxic drugs. Non-selective or selective adenosine receptor agonists were administered to the mice in protective or treatment regimens. Hematopoietic status of the animals was assessed by a complex evaluation comprising parameters of hematopoietic progenitor and precursor cells, as well as of peripheral blood cells.
Results: Non-selective activation of adenosine receptors has been achieved by concommitant administration of adenosine monophosphate (AMP), an adenosine prodrug, and dipyridamole (DP) which prevents cellular uptake of adenosine and potentiates, thus, its receptor-mediated action. This drug combination has been found to support hematopoiesis both in normal and radiation- or chemotherapy-exposed mice. DP+AMP combination has been also observed to potentiate hematopoiesis-stimulating effects of granulocyte colony-stimulating factor. Selective activation of adenosine A_1 receptors by N^6-cyclopentyladenosine inhibited whereas that of A_3 receptors by N^6-(3-iodobenzyl)adenosine-5'-*N*-methyluronamide (IB-MECA) stimulated proliferation of hematopoietic progenitor cells. IB-MECA has been found to support hematopoiesis in γ-irradiated mice.
Conclusions: Elevation of extracellular adenosine leading to non-selective activation of adenosine receptors stimulates hematopoiesis. This effect can be utilized in the treatment of myelosuppression of various origin, as shown in animal experiments. Utilization of synthetic adenosine receptor agonists, selective for individual adenosine receptor subtypes, has revealed that adenosine A_3 receptor activation is responsible for the earlier described action of elevated extracellular adenosine. These findings may be of significance also in clinical practice.

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Heterocyclic hydrazones induce radical formation and dissipation of mitochondrial membrane potential

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Background: The novel compound *N*-benzoxazol-2-yl-*N'*-1-(isoquinolin-3-yl-ethylidene)-hydrazine (EPH136) has been shown to exhibit antitumor activity *in vitro* and *in vivo*. A COMPARE analysis showed that the patterns of cellular effects of EPH136 are not related to any of 175 standard antitumor agents with a known mechanism of action.
Methods: In order to help identify the mechanism of action we employed the following methods: (i) a bioinformatics approach, called partial least squares modelling in latent variables, (ii) a DNA microarray for detection of the expression of ~5000 known genes following treatment of HT-29 colon carcinoma cells with a two-fold IC_{50} concentration of EPH136, (iii) detection of radicals by luminol, (iv) determination of the mitochondrial membrane potential by JC-1 and by TMRM, (v) treatment of cells with EPH136 and the radical scavenger N-acetylcysteine and (vi) treatment of cells with elevated levels of glutathione.
Results: The 60 genes found by the bioinformatic approach to be most important for the antiproliferative effect of EPH136 are involved in nucleoside, nucleotide, nucleic acid binding and metabolism, developmental processes, protein modification and metabolism. The genes that were up-regulated more than two-fold (in DNA microarrays) compared to untreated controls belong to the same classes as found by the bioinformatic approach. Many of these proteins are regulated by oxidation/reduction and so we concluded that formation of radicals may be involved in the mechanism of action. We found that EPH136 leads to generation of radicals, swelling of mitochondria and dissipation of the mitochondrial membrane potential. The antiproliferative activity of EPH136 was prevented by the radical scavenger *N*-acetylcysteine. Cells with elevated glutathione exhibited resistance to EPH136.
Conclusions: The mechanism of action of the novel experimental anticancer drug EPH136 is generation of radicals and dissipation of the mitochondrial membrane potential.

Alpha1-Adrenoceptor-Mediated Pronociception Attenuates Induced Analgesia in Rat

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Background: Unrelieved pain is a worldwide problem. Understanding mechanisms of endogenous pain circuits may identify areas that interfere with clinical pain relief. The A7 catecholamine cell group provides the majority of noradrenergic input to the spinal cord dorsal horn in some rat strains. Stimulation of the A7 cell group produces antinociception blocked by alpha2-adrenoceptor antagonists. This finding forms the basis in part for clinical use of alpha-adrenergic agonists and reuptake inhibitors as analgesics. Yet alpha-adrenergic agents are not always effective and have major side effects. We report several studies in which we stimulated the A7 cell group directly with morphine sulfate, or indirectly by stimulating the hypothalamus, and obtained pronociception mediated by alpha1-adrenoceptors that attenuates alpha2-mediated antinociception.
Methods: In the first study, morphine sulfate (5 or 10 ug) was microinjected in the A7 cell area in lighty anesthetized female sprague-dawley rats, followed by intrathecal (IT) injection of either the alpha1 antagonist WB4101, the alpha2 antagonist yohimbine (97 nmol), or saline for control. Response latencies were measured using the tail flick and foot withdrawal tests. In subsequent studies the lateral (LH) or the posterior (PH) hypothalamus was stimulated using carbachol and IT alpha-antagonists given as described above.
Results: The 5 ug dose of morphine decreased foot and tail withdrawal latencies, a hyperalgesic response. IT injection of yohimbine did not alter this hyperalgesia, but WB4101 reversed the hyperalgesia and produced antinociception. Microinjection of 10 ug morphine in the A7 area did not alter nociceptive responses. However, IT injection of yohimbine produced significant pronociception, while WB401 produced significant antinociception. The LH and PH project to the A7 cell group and do not contain spinally-projecting noradrenergic neurons. Stimulation of either the LH or PH produced antinociception blocked by IT yohimbine but increased by IT WB4101.
Conclusions: Stimulating the A7 cell group produces bidirectional control of nociception in which alpha1-adrenoceptors increase nociception while alpha2-adrenoceptors inhibit nociception. These findings indicate that therapies involving the descending alpha-adrenergic system may be less effective if alpha1-adrenoceptors are activated along with alpha2-adrenoceptors.

Growth Hormone: how to catch the magic bullet of the modern athlete

HOLT RIG on behalf of the GH-2004 project team

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There is widespread anecdotal evidence that growth hormone (GH) is used by athletes for its anabolic and lipolytic properties. There has been considerable debate, however, whether GH has a performance enhancing effect in healthy adults. Although clinical trials are not best placed to address this issue because of the small margins of victory in many athletic events, recent studies have shown that GH can improve physical fitness as well as body composition.

Despite GH appearing on the World Anti-Doping Agency list of banned substances, the detection of abuse with GH is challenging because unlike many substances of abuse, such as synthetic anabolic steroids, GH is a naturally occurring substance. Demonstration of exogenous administration must rely on detecting concentrations exceeding normal physiological circumstances. Two approaches have been developed to detect GH abuse. The first relies on the measurement of pituitary GH isoforms. When rhGH, which contains only 22kD GH, is administered in sufficiently high doses, there is a suppression of endogenous GH secretion and therefore the ratio between 22kD GH and total GH is altered. The second method is based on the measurement of markers of GH action. After evaluation of 25 potential markers, the GH-2000 team proposed a method using insulin like growth factor-I (IGF-I) and type 3 pro-collagen (P-III-P). The administration of rhGH leads to a significant rise in these markers, the magnitude and duration of elevation of which is dependent on the rhGH dose and gender. As a result GH abuse can be detected with reasonable sensitivity and specificity.

The piggy back approach or how to find new lead structures for antiinfective or antiviral drugs

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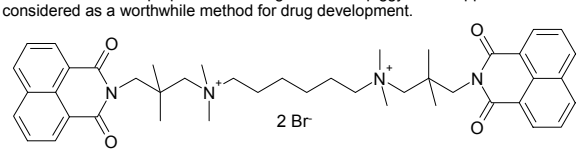
Background: Mono- and bisnaphthalimides such as amonafide and elinafide have been developed as cancerostatic drugs by Brana et al. [1]. Recently, naphthalimides could be profiled as potent ligands of the muscarinic receptor.
Methods: Within the frame of a broad screening program for drugs a small library of recently synthezised mono- and bisquartary naphthalimides were tested for their activity against *Trypanosoma brucei* [2], *Leishmania major*, *Candida albicans*, *Staphylococcus aureus*, *Plasmodium falciparum,* and Measle and Nipah virus as well as for toxicity against macrophages.
Results: Depending on the substitution pattern the mono- and bisnaphthalimides were active against either microorganism in the lower micromolar and nanomolar range of concentration combined with almost no cytotoxicity.
Conclusion: The bisnaphthalimides studied here are perfect lead compounds for further drug development. Interestingly, the structure-activity relationships were different for each purpose indicating that the piggy-back approach can be considered as a worthwhile method for drug development.



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Inhibitors of Serine/Threonine Protein Phosphatases at the Dawn of a Clinical Era.

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The reversible phosphorylation of proteins regulates the biological activity of many diverse cellular processes, including gene transcription, protein-protein interactions, cell-cycle progression and apoptosis. Phosphorylation occurs principally on ser/thr and tyr residues, with the addition and removal of phosphate catalyzed by kinases and phosphatases, respectively. Although protein phosphatases were once viewed as simple house keeping proteins, in the last decade it has become eminently clear that they are actually dynamic and highly regulated enzymes. Therefore, the development of compounds that alter the activity of “key” phosphatases is rapidly emerging as an important area in drug discovery. Because the majority of protein phosphorylation (>90%) occurs on serine and threonine residues, the identification of specific agents that alter the activity of key ser/thr phosphatases seems especially promising for development. For the PPP-family of phosphatases, several lead compounds for drug development have come from studying the biological actions of natural products, such as fostriecin and cantharidin, which was identified as an activate constituent in a Chinese beetle extract developed over 1500 years ago for the treatment of human cancer. The current challenge for further drug development is to identify “key” phosphatases and more selective inhibitors, because non-selective inhibitors, such as calyculin A, microcystins and nodularin are highly toxic to both normal and cancer cells. The high-resolution crystal structures of PP1, PP2A and PP5 have been solved, and structure/function studies with derivatives of highly selective inhibitors (i.e. fostriecin and cytostatin A) provide insight into methods for developing novel inhibitors, as well as challenges that must be overcome for small molecule development. The development of siRNA and antisense oligonucleotides that support RNAase H mediated degradation of the targeted mRNA has resulted in compounds capable of specifically suppressing the expression of each ser/thr phosphatase in human cells. Such compounds have already proven useful for the validation of drug targets, and if difficulties associated with in vivo stability for siRNA or systemic delivery of antisense oligonucleotides can be overcome, these compounds are poised to have a major impact on the clinical management of many human disorders.

What Sort of Light Is At the End Of the Tunnel In Anti-Allergic Drugs?

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Background: Studies on the efficacy of anti-allergic drugs in the treatment of allergic rhino-conjunctivitis have yielded inconsistent and unsatisfying results, notwithstanding their approval for this indication.
Objective: The objective of this study was to evaluate and compare the therapeutic effect of approved anti-allergic drugs as well as compounds under development to placebo in subjects with allergic rhino-conjunctivitis.
Methods: In consecutive single-centre, double-blind, placebo controlled, cross over studies, more than 500 subjects with confirmed allergic rhinitis due to grass pollen or house dust mite were randomised to receive active or placebo drugs and exposed to the accordant allergen (grass or mite) in the Vienna Challenge Chamber (VCC) for several hours. Patients recorded symptoms at 15-minute intervals, nasal secretion and nasal resistance were evaluated every 30-minutes. The primary endpoint was the mean change from placebo in total symptom score.
Results: All of the investigated compounds like H1-receptor antagonists, topically used steroids and other mediator antagonists were significantly different from placebo in the primary endpoint but not very different in their therapeutic power. However, all of the approved drugs did not reach a therapeutic potency of more than 35% over placebo reflecting the frustration of many patients suffering from allergic rhinitis. Never the less, some compounds, out of the pipeline, have overcome this threshold, whereas others reaching the target were discontinued in their development, due to the risk/benefit viewpoint.
Conclusion: The standardised and validates model of an allergen challenge chamber like the VCC enables us to compare results from different trials on a historical basis. Albeit approved anti-allergic drugs do not fully satisfy suffering individuals, several new compounds are under investigation and hopefully reaching a risk/benefit ration which will be acceptable for the patients.

Antitumor Effect of a Novel NF-κB Targeting Therapy in Bladder Cancers

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Background: Nuclear factor (NF)- κB is a transcription factor not only induces and controls various genes including inflammatory cytokines, but also activates genes with antiapoptosis, tumor proliferation and invasion. It has been clearly demonstrated that certain advanced human bladder cancer cells constitutively acquire the ability to activate NF-κB, which not only protects cancer cells from apoptotic cell death, but also up-regulates the production of various cytokines that might induce malignant potential of the disease. NF-κB activation inhibitor, therefore, might be useful as novel anticancer agents. We have designed and synthesized a NF-κB inhibitor, dehydroxymethylepoxyquinomicin (DHMEQ) and investigated the effectiveness of DHMEQ against advanced human bladder cancer cells, KU-19-19 in which NF-κB is constitutively activated.
Methods: KU19-19 was implanted s.c. in the flank of nude mice. Daily i.p. administration of 2 mg/kg DHMEQ was started from 7 days after tumor implantation. After 28 days, mice were sacrificed and all the tumors were evaluated. Microvessels in tumor specimens were counted after immunostaining with an anti-CD34 monoclonal antibody. Apoptosis was measured by TUNEL assay using Apoptosis *in situ* Detection Kit. The average number of positively stained cells was counted and apoptosis index was calculated.
Results: Inhibition of NF-κB by transfection of adenovirus vectors expressing stable form of NF-κB inhibitor, IκBα, inhibited KU19-19 cell growth and induced apoptosis. DNA binding activity of NF-κB was completely inhibited by DHMEQ. Marked levels of apoptosis were observed after DHMEQ administration. DHMEQ treatment *in vivo* inhibited KU19-19 tumor growth. Tumor volume after DHMEQ treatment was 3110±945 cmm vs 6019±2309 cmm of control mice ($P<0.05$). A statistically significant decrease in MVD in DHMEQ-treated tumors was observed. Blood vessels in the tumors derived from control mice showed well-developed vascular networks. In contrast, the vessels in the tumors of DHMEQ-treated mice consisted of poorly developed networks. The apoptotic index was increased 2.3-fold in DHMEQ-treated tumors than control.
Conclusions: Targeting NF-κB could be a new strategy of treatment against advanced bladder cancer.

The Clinical Pipeline of a Candidate Malaria Vaccine that Targets the Achilles' Heel Antigen of *Plasmodium falciparum*

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Background: Malaria remains as one of the most infectious disease globally. Failure of existing control strategies necessitates the need for vaccine development. We focused on the development of an effective vaccine using a recombinant SE36 protein based from the N-terminal domain of Serine Repeat Antigen (SERA5) of *Plasmodium falciparum*.
Methods: *In vitro* studies have elucidated the transcription profile, processing and localization of SERA5. A recombinant version of the 47 KDa N-terminal domain, and a modified version with high hydrophilicity (SE36), has been produced in *E. coli*. Molecular and immunology based epidemiological studies have been conducted in Uganda and Solomon Islands. An efficient fermentation and purification process has also been developed for the production of this antigen in a scale comparable with industrial manufacture and this has been used for preclinical and toxicology studies in animals to demonstrate safety and immunogenicity. The candidate vaccine, BK-SE36, was formulated adsorbed to aluminum hydroxide gel and is manufactured as a lyophilized product under Good Manufacturing Practices (GMP). A phase 1a clinical trial has been conducted in Japan.
Results: *In vitro,* several lines of evidence suggest that SERA5, which belongs to a multigene family unique to *Plasmodium,* plays an essential role in parasite development and merozoite egress. Immunoepidemiological data underscores the uniqueness of SERA: naturally induced antibody response to SE36 protein correlated with increased protective immunity in adults and children. Higher levels of anti-SE36 IgG3 titer were associated with the absence of fever and lower parasitemia in children under 15 years; and were associated with protection against severe malaria in children under 5. Sero-conversion rates were 50% or less in >16 yr-old; and less than 10% in <10 yr-old individuals. Preclinical studies in animals showed that BK-SE36 was safe and highly immunogenic. Immunological test using squirrel monkeys provided significant protection after *P. falciparum* challenge infection; and antibody titers were significantly boosted. BK-SE36 was, likewise, safe and highly immunogenic in chimpanzees. No significant safety issues have been identified in healthy, malaria-unexposed adults in a Phase Ia clinical trial in Japan. Cumulative data confirms the potential of this candidate vaccine.

The success story of Japanese DTaP control and a further development of vaccine evaluation

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Background: Different from therapeutic drugs, vaccines for controlling infectious diseases are given to majority of healthy population and, in most cases, need booster immunizations. However, many procedures applied to vaccine evaluation particularly for licensure are specific for therapeutic drugs and need to be revised.
Methods: Adverse Event Following Immunization (AEFI) cases before and after implementation of diphtheria tetanus acellular pertussis vaccine (DTaP) were compared. Acellular pertussis based combination vaccnes imported from foreign markets were compared with Japanese DTaPs for injection site injuring effects in mouse footpad swelling, rabbit skin swelling and mouse quardriceps muscle injection models. Regarding local reaction to a booster dose, mice were intramuscularly immunized with a vaccine sample twice at one month interval and challenged at footpad two weeks later with 50 microlitre of diphtheria toxoid to measure swelling reaction.
Results: Annually two mortality and three encephalopathy cases, in average, were reported among five million doses of diphtheria tetanus whole cell pertussis vaccine (DTwP). However, report of such severe AEFI has become quite rare after 1991 when minimum requirements of biological products (MRBP) for DTaP was revised to strengthen detoxification of pertussis toxin (Table 1) to suggest relevance of DTwP with the rare but severe AEFI.

Vaccine	Year	Encephalopathy	(Death)	Shock to Death	Total dose
wP/DwP/DTwP	1952-1974	62	28	17	Ca. 110,000,000
DTaP	1995-2000	1	1	0	28,671,071

Regarding local reaction, all imported vaccines induced very strong inflammation and tissue injury at injection sites of mouse footpad, rabbit skin and mouse quardriceps muscle while Japanese DTaP induced no such reaction to suggest difference in tissue damaging effect of the vaccines. We evaluated enhancing effect of DTaP with varied levels of residual PT activity on local reaction to a booster dose using mouse footpad swelling model. A significant correlation was seen between mouse footpad swelling and residual PT activity of immunized vaccine batches.
Conclusions: 1) Reduced toxicity in laboratory tests seemed to be relevant to safety. 2) Clinical observation could not evaluate injection site injury. 3) DTaP may enhance local reaction to booster doses. 4) Not only clinical evaluations, laboratory models need to be focused on in vaccine evaluation.

Characterization of an Active Pharmaceutical Ingredient by Its Dissolution Properties: Amoxicillin Trihydrate as a Model Drug

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Background: An ordinary powder sample of an active pharmaceutical ingredient (API) is composed of particles having various properties with respect to their shapes, dimensions, etc. When taking into account such differences in particles, the dissolution of a sample can be described using a dissolution theory of heterogeneous particle populations, where every population is characterized by a rate coefficient (α), consisting of a geometric factor and a material constant. Aims: 1) To develop a requested theory as a system of differential equations (SDE's). 2) To prove the applicability of the theory for sieved fractions of API. 3) To predict dissolution profiles for samples of known particle size distribution. 4) To find a coherence between the developed and published theories.
Methods: The introduced SDE's was used for 120 dissolution profiles of sieved amoxicillin trihydrate samples, which were assumed to be two-population systems, by numerical solutions of the SDE's and the "Solver" function of Microsoft® Excel. The calculated and measured curves were compared by factors of difference (f_1) and similarity (f_2). The dependence of the dissolution rate coefficients on dose, particle size and rotation speed of the paddles of the dissolution device was established by factorial analysis.
Results 1959 data pairs of 120 measured and calculated profiles highly correlated ($R^2 = 0.9997$), while the average (minimum, maximum) of f_1 and f_2 is 0.76 (0.09, 3.42) and 96.91 (80.33, 99.91) respectively. The factor analysis of dissolution rate coefficients indicated that the dissolution rate α_1 depends only on rotation speed ($p < 0.0001$), while α_2 depends solely on the dose ($p = 0.0058$). Hence, α_1 is a dissolution rate constant of real particles, whereas α_2 expresses a rapid disintegration of aggregates of smaller particles. The dissolution profile of a mixed API sample (18 differential equations) was successfully predicted ($f_1 = 2.8$; $f_2 = 86.0$).
Conclusions: 1) The developed theory is able to describe the dissolution of heterogonous particle populations. 2) Based on a known sample of particle size distributions, a dissolution profile can be predicted. 3) By solving the introduced SDE's under certain conditions, the theories developed by Hixon and Crowell, Noyes and Whitney, Niedergall and Goyan, and Pothisiri and Carstensen can be derived, indicating a coherence with the known theories.

Estradiol as Membrane Targeting Modulator of Neuronal Cell Functions

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Background: Accumulating evidence from basic science studies demonstrates that estrogens exert profound protective actions against various forms of neurodegenerative diseases and injury. Recently, evidence has accumulated in favour of membrane effects of estrogens due to either a change in some membrane properties or to specific binding to membrane targets. One examples of estrogen modulation of membrane properties involve the modulation of intracellular calcium homeostasis. In our earlier as well as recent-nonpublished study both modulation of intracellular calcium stores and regulation of plasma membrane calcium channels have been proposed. We examined *in vitro* effects of 17β-estradiol (E2) on intracellular calcium ($[Ca^{2+}]_i$) concentration in isolated presynaptic nerve terminals (synaptosomes) from whole female rat brain and discrete brain regions by measuring dose-dependent effects of E2 on 1) Ca^{2+} influx in synaptosomes through voltage-gated channels and extrusion of Ca^{2+} by sodium/calcium (Na/Ca) exchanger, 2) effects of E2 on Ca^{2+} influx in synaptosomal mitochondria through uniporter and release of Ca^{2+} through mitochondrial Na/Ca exchanger. To evaluate the mechanisms of E2 action we measured its specific binding to isolated membrane of synaptosomes (SPM) and synaptosomal mitochondria, and defined binding characteristics.
Methods: Synaptosomes were isolated from whole brain (WB), brain stem (BS), nucleus caudatus (NC) and hippocampus (Hip) of chronicaly ovariectomized three month-old female rats (18 animals/brain structure/experimet). Binding of E2 to isolated synaptosomal plasma membranes (SPM) and synaptosomal mitochondria were calculated by subtracting non-specific bound (in the presence of 100-fould excess unlabeled E2) from total bound of ^{3}H-labeled E2 (10^{-10} - 10^{-7} M). For Ca^{2+} flux measurement synaptosomes were preincubated in the presence or absence of E2 (10^{-9} - 10^{-7} M) for 15 min. The voltage-dependent $^{45}Ca^{2+}$ influx were measured in the presence of 50mM KCl and the Na-dependent Ca^{2+} efflux in resting conditions (4 mM KCl) for 30 sec. Retained $^{45}Ca^{2+}$ in the synaptosomes were determined by radioactivity measurement after filtrating synaptosomal suspension through nitrocellulose filters 0.45 μm pore size. Synaptosomal mitochondria were preincubated with or without E2 (10^{-12} - 10^{-7} M) for 10 min and $^{45}Ca^{2+}$ influx through uniporter as well Na-dependent Ca^{2+} extrusion in the $^{45}Ca^{2+}$ preloaded mitochondria in presence of 20 mM NaCl and 0.2 mM EDTA were measured.
Results: E2 bound to two specific sites on SPM from NC ($Bmax_1$ 0.68 pmol/mg, Km_1 26 nM and $Bmax_2$ 0.16 pmol/mg, Km_2 3.6 nM), Hip ($Bmax_1$ 0.88 pmol/mg, Km_1 30 nM and $Bmax_2$ 0.07 pmol/mg, Km_2 2.6 nM) and BS ($Bmax_1$ 0.3 pmol/mg, Km_1 26 nM and $Bmax_2$ 0.06 pmol/mg, Km_2 4 nM) and one binding site in WB (Bmax 2 pmol/mg, Km 40 nM). In synaptosomal mitochondria we detected one specific binding site in NC (Bmax 48 pmol/mg, Km 32 nM), Hip (Bmax 17 pmol/mg, Km 17 nM) BS (Bmax 3.4 pmol/mg, Km 1.8 nM) and WB (Bmax 0.05 pmol/mg, Km 0.46 nM). E2 at concentrations up to 10^{-8} M increased whereas at higher concentrations decreased voltage-dependent Ca^{2+} influx in synaptosomes of all brain structures, while at concentrations $5x10^{-9}$ and 10^{-8} M increased Na-dependent efflux of Ca^{2+} in NC and Hip. Mitochondrial influx of Ca^{2+} was not affected by E2 while physiological concentrations of E2 decreased mitochondrial Na-dependent efflux.
Conclusions: E2 at physiologial concentrations specificaly bound to SPM and synaptosomal mitochondria from discret brain regions and at same concentrations modulate $[Ca^{2+}]_i$ by affecting voltage-dependent influx and Na-dependent extrusion of calcium in synaptosomes and decreasing mitochondrial Ca release.

Nongenomic Corticosteroid-adrenergic Drug Interactions in the Airway

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Background: Organic cation transporters (OCTs) have an important role in tissue distribution and elimination of cationic drugs. Carrier-mediated disposal of cationic bronchodilators in the airway tissue, however, is incompletely understood. Aim: To assess the uptake of long-acting β_2-agonist bronchodilators by bronchial and vascular smooth muscle cells (SMCs).
Methods: Human airway cells and tissues obtained from organ donors were evaluated for cationic drug transporter expression by quantitative RT-PCR and immunofluorescence. For *in vitro* functional studies, [^{3}H]-formoterol (FORM) and [^{3}H]-salmeterol (SALM) uptake by bronchial and vascular SMCs was measured.
Results: RT-PCR analysis indicated high mRNA levels for the corticosteroid-sensitive OCT3 in bronchial and vascular SMC. Immunofluorescence staining of airway sections confirmed OCT3 expression in these cells. In bronchial SMC, uptake of the cationic FORM was inhibited with OCT inhibitors. Corticosteroids also inhibited FORM uptake through a rapid (within 15 min) nongenomic action, with the following rank order (relative potency): *des*-ciclesonide (11.1) > hydrocortisone (5.2) > budesonide (3.8) > beclomethasone 17-monopropionate (1.8) > beclomethasone dipropionate (1.7) > ciclesonide (1.4) > fluticasone (1). The corticosteroid-induced inhibition was significantly higher in vascular than bronchial SMCs. In comparison to FORM, uptake of the noncharged lipophilic SALM was about 10-fold higher (28.4 ± 1.7 vs. 327.5 ± 13.7 pmol/mg/15 min; $p < 0.05$), and insensitive to OCT inhibitors and corticosteroids.
Conclusions: Our findings suggest that corticosteroids, through OCT3 inhibition, rapidly interfere with drug disposal mechanisms in the airway. Increased tissue retention of inhaled cationic bronchodilators due by the corticosteroid-sensitive disposal mechanism could acutely improve bronchodilator responses. This novel immediate interaction supports the use of such combinations in asthma therapy.

Dioxin like compounds for anti-infective agents of know as its degrading enzyme from its resistant *Geobacillus midousuji* thermophile

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Background: Dioxins are mostly created by human activity and can be classified in the family of halogenated organic compounds, have been shown to bioaccumulate in humans.The highest toxicity is the 2,3,7,8- tetrachloro dibenzo - 1,4- dioxin (TCDD). We have noticed Dioxins are kinds of anti-inflamatory and anti-proliferative agents through its resistant bacteria with its respiratory activity.
Materials and Methods: The fibroblast was given from Dr. Emiko Sano of Proteios Laboratory (Yokohama, Japan) of human forehead epithelial primary culture. MIC assay is designed for 2,000 cells /100uL a well. After 24 hours pre-culture, serum was depleted as starving culture in 30 hours. In conditioned medium of TCDD and solvent DMSO, cells were cultured in 18 hours, then luciferin and luciferase RLU(relative light unit) was measured by Luminometer.
Results: MIC of TCDD by human cell viability assay is estimated as 1pg/mL by the Government.

	TCDD				
RLU	starving	0.001fg/mL	10fg/mL	100fg/mL	1pg/mL
1	389990	501220	232120	295350	242420
2	358090	330230	270460	248020	232350
3	358820	309620	453600	252030	250770
av.	368967	380357	318727	265133	241847

2000cells /100uL/well

In this study MIC of ATP production showed more sensitive result around 10-100fg/mL.
The reductive enzyme has been unique glutathione S transferase of *B. midousuji*.
Conclusions : TCDD has inhibitory activity of cellular ATP production, as new anti-inflammatory, anti-proliferative compound with core skeletal structure.

Acknowledgement: This study was granted by Ministry of Environment of Japan.

The prevalence and the resistance mechanism of floroquinolones in bacteria isolated from Bangladesh

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Background: Antimicrobials have been a much misused product in the world. In Bangladesh only 8% of the total used antibiotics are prescribed by a physician; and in poultry, veterinary and aquaculture are using unknown quantities of large amount antibiotics in an uncontrolled manner. This gives rise to the emergence of antibiotic-resistant bacteria. Our group has been working on understanding the origin of antibiotic resistance, resistance mechanism and route of spreading in Bangladesh. Here, we report contribution of medical college hospitals for originating antibiotics-floroquinolones resistant bacteria and its mechanism of actions.
Methods: To address the problems, we studied following parameters in hospitals wastewater (HWW)- i. correlation of antibiotics used by the patients and resistant bacteria development; ii. effect of wastewater on sensitive bacteria; and iii. determination of floroquinolones resistance mechanisms using DNA-sequencing technology.
Results: The bioassay using sensitive *Escherichia coli*^{s} clearly revealed that HWW contained active antibiotics at higher concentration than MIC_{50} for sensitive bacteria. Enumeration of total resistant bacterial count showed that the count was about 5-log higher in HWW. We randomly selected 52 *E. coli* isolates with high multi-drug resistance including ciprofloxacin (>MBC_{100} 600 μg/mL). DNA sequencing data revealed that ciprofloxacin resistance in bacteria occurs due to acquisition of mutations in *gyrA* gene. Computer modeling of the mutant and wild-DNA gyraseA based on available DNA gyraseA crystal structure suggests that acquisition of double mutation that leads to alteration of the ciprofloxacin binding pocket may be the reason of high resistance properties shown by the isolates.

Sample	Total viable Bacteria per mL	Total viable count over control	Total resistant Count per ml	Percentage of resistant
HWW	1.136×10^8	3.55	1.54×10^7	13.54
Control	3.20×10^7	1.0	9.8 x 10	0.0003

Conclusions: In conclusion, our findings clearly demonstrate that – i. HWW have ecotoxicological effect in spreading resistant bacteria as well as active antibiotics in the environments; and ii. floroquinolones resistance in bacteria isolated from Bangladesh are due to acquisition of mutation in *gyrA* gene rather plasmid born.

Targeting CLEC5a/MDL1-1 for he Treatment of Dengue Hemorrhagic Fever

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Background: Members of non Toll-like receptors (NTLRs) have been demonstrated as pattern recognition receptors to bacteria and fungi, such as mannose receptor and dectin-1 receptor, respectively. However, the ligands and the functions of most of NTLR, including C-type lectin, C-type-like lectin, Ig-like receptors [TREM and TREM-like transcript (TLT)] are still unknown.
Method: The extracellular domain of 22 NTLRs expressed on myeloid lineages were cloned by RT-PCR, and was fused with the Fc portion of human IgG1 as fusion gene. After expression in eukaryotic system, the fusion proteins were immobilized on ELISA plates to capture dengue virus
Results: Three C-type lectin receptors—DC-SIGN, DC-SIGNR, and CLEC5A/MDL-1—, are able to interact with dengue virion (DV) directly. Further study indicates that DV triggers both CLEC5A and TLR7/8 to produce proinflammatory cytokines synergistically, but CLEC5A is not involved in the production of interferon-alpha (IFN-α). Incubation of macrophages (MΦ) with DV induced proinflammatory cytokines release, and blockade of DV-CLEC5A interaction by shRNA or antagonistic mAb attenuates proinflammatory cytokine release without suppressing IFN-α secretion in vitro. We established an murine model system to induce dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), and we found that antagonistic anti-antibody inhibits plasma leakage and increases the survival rate of mice infected by DV.
Conclusion: In addition to Toll-like receptors, the NTLRs are the promising candidates involved in recognition of pathogen-associated molecular patterns. Our results demonstrates that virions acts as a ligand to activate macrophage via surface receptor, and blockade of NTLR-virion interaction might become a novel strategy to prevent dengue hemorrhagic fever as well as to decrease the mortality of patients suffered from other viral infections in the future.

The Role of Metabolism, Pharmacokinetics and Mass Spectrometry in Selection of a Thrombin Receptor Antagonist for Development

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The rising costs and time associated with bringing new medicines to the market have created a need for a new paradigm for reducing the attrition rates of drug candidates in both preclinical and clinical development stages. Early appraisal of drug metabolism and pharmacokinetic (DMPK) parameters is now possible due to several higher throughput in vitro and in vivo screens. This knowledge of DMPK properties should not only shorten the timelines for the selection of drug candidates but also enhance the probability of their success for development. The role of DMPK researchers in the drug discovery paradigm should not be limited to screening a large array of compounds during the lead optimization process but should strive for an understanding of the absorption, distribution, metabolism, excretion, and potential drug-related toxicities of a chemical series. As an example, in this presentation the role of DMPK and mass spectrometry-based techniques in support of the Thrombin Receptor Antagonist program will be presented.

Anti-neoplastic properties of tea catechins are associated with pro-differentiation caspase 14 gene expression: implementation for novel therapies

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Background: Exploration of novel approaches with innovative therapies is needed to combat epithelial cancer. We previously reported that caspase-14, a gene expresses during terminal differentiation of certain epithelial cells, is induced by green tea polyphenols. Our hypothesis is that the expression of caspase 14 induces tumor cell death without damaging normal epithelial cells. Therefore, caspase 14 is a suitable candidate for novel gene therapy to treat a variety of cancers.
Objectives: to express exogenous human caspase 14 in human epithelial cancer cells (OSC2) by plasmid transfection and adenovirus delivery, and determine the effects of caspase-14 expression on cell growth, cell death, and tumorigenicity.
Methods: The human cancer cell lines A431, HSG, and OSC2 were either transfected with caspase 14 expressing plasmid, or infected by adenovirus expressing caspase 14 cDNA. Expression of caspase 14 was confirmed by Western blotting. Cell morphology was monitored by microscopic photography, cell growth was measured by cell counting and BrdU assay, and cell viability was determined by MTT assay. In addition, the cancer cells were xenografted into athymic mice to determine the tumorigenicity.
Results: expression of caspase-14 induced an undefined cell death in these cancer cells compared to the control cells. Cell growth and cell viability were inhibited significantly by caspase-14 expression. Xenograft of caspase-14-expressing cancer cells into athymic mice resulted in significantly reduced tumorigenicity, which could due to an inhibitory effect of caspase 14 on tumor vascularization.
Conclusions: human epithelial cancer cells undergo growth inhibition and cell death when exogenous caspase-14 was expressed in these undifferentiated tumor cells. Caspase-14 expression in these cells also reduced tumorigenicity *in vivo*. Further effort is warranted to explore if caspase 14-expressing adenovirus could be used as a potential therapeutic approach to treat human cancers.

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Development of species-specific STAMPs (specific targeted antimicrobial peptide) that target and kill only *Streptococcus pneumoniae* within a polymicrobial community

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Background: STAMP technology uses a tripartite polypeptide with two functional moieties: a killing moiety made of a nonspecific antimicrobial peptide; and a targeting moiety made of a species-specific binding peptide that are joined together by a linker peptide that provides free rotation. The targeting moiety provides specific binding to a selected pathogen and facilitates the targeted delivery of the attached antimicrobial peptide which significantly enhances its killing kinetics compared to the untargeted antimicrobial peptide. STAMPs developed against *S. mutans* and *Pseudomonas aeruginosa* have high microbiocidal activity against their targets, but low or no activity against other microbes
Methods: Using data from the pneumococcal core and supragenomic analyses performed at the CGS we identified several pneumococcal-specific surface-exposed protein targets including the competence stimulating peptide (CSP) receptors and the BIps. Using bioinformatic approaches a dozen candidate *Sp*-specific targeting peptides were designed to interact with these targets, and were then synthesized and tested for binding against multiple bacterial species
Results: One of these peptides, a truncated form of *Sp*'s CSP, binds very avidly to *Sp* biofilms, but does not show any binding to *H. influenzae*, a major co-colonizer of the nasopharynx, nor does it bind to the related *S. mutans* to any appreciable degree. Another *Sp*-specific peptide (Sp687) specifically binds to both *Sp* biofilms as well as planktonic bacteria. Out of 12 Sp-encoded peptide pheromones and related peptides identified using comparative genomics, 7 gave binding results equivalent to or better than the CSP and Sp687 peptides.
Conclusions: The ability of these targeting peptides to bind to biofilm envirovars is of key importance as they represent the major target in chronic infections. By combining the antibiofilm affects of the previously characterized antibacterial peptides with the specificity of the pneumococcal targeting peptides we will be able to construct an anti-*Sp* biofilm magic bullet.

Opening of Blood-Brain Tumor Barrier by Phosphodiesterase Type 5 (PDE5) Inhibitors in a Mice Metastatic Brain Tumor Model

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Background: While adequate delivery of drugs may occur in systemic tumors, the blood-brain tumor barrier (BTB) limits delivery of anti-tumor agents into brain tumors including metastases. In this study, we examined the role of phosphodiesterase type 5 (PDE5) inhibitors in BTB opening in a mice metastatic brain tumor model.
Methods: Firstly, we established the metastatic brain tumor model by implanting CRL-5904 cells (human brain metastasis of non-small cell lung cancer) intracranially and flankly. The mice were treated with different tracers with or without vardenafil (Levitra), an inhibitor of cGMP-specific PDE5. Then, we detected the *in vivo* BTB permeability by using fluorescence microscopy, quantitative autoradiographic method and Xenogene imaging system. We also investigated the in vitro drug uptake by using the transwell system to mimic blood brain barrier.
Results: We showed that oral administration of vardenafil significantly increased doxorubicin uptake in brain tumors. Further, vardenafil could increase the tumor permeability of therapeutic antibody Herceptin and big molecule tracer ^{14}C-Dextran intracranially and flankly, but not in contralateral normal brain. *In vitro* drug uptake assay demonstrated that vardenafil enhanced the uptake of doxorubicin in both CRL-5904 and human brain microvessel endothelial cells (HBMEC). Sildenafil (Viagra) increased not only the uptake of the tracer ^{14}C-sucrose but also that of the chemotherapeutic drug ^{14}C-carboplatin in brain microvessel endothelial cells and tumor cells. Furthermore, filipin, an inhibitor of caveolae endocytosis pathway, could block PDE5 inhibitor-induced drug uptake, suggesting a mechanism via caveolae transcytosis pathway.
Conclusions: These findings suggest that PDE5 in metastatic brain tumors may serve as an effective target for pharmacological modulation of BTB permeability to enhance selective delivery of chemotherapeutic drugs to metastatic brain tumors.

Novel Targeted Anticancer Prodrugs of Phosphoramide Mustard

HU L

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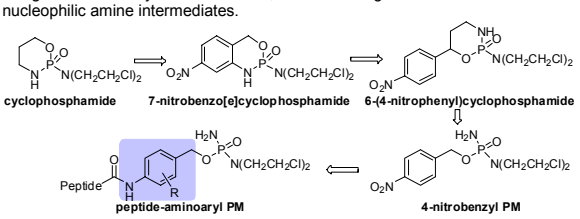
Background: Many anticancer agents in current clinical use lack the desired tumor-selectivity and are associated with systemic side effects. Prodrug design is one approach that could potentially improve the target-selectivity of anticancer agents. Efforts in our laboratory have been focused on designing analogs of cyclophosphamide and phosphoramide mustard (PM) to move the site of activation from the liver to tumor tissues through the incorporation of reductive and proteolytic triggers.
Methods: A series of nitroaromatic analogs and peptide aminoarylmethyl conjugates was designed to efficiently release the active phosphoramide mustard in a site-specific manner in tumor tissues, thus increasing the selectivity in killing cancer cells and decreasing systemic toxicity. Traceless linkers have been designed and optimized to efficiently release the active phosphoramide mustard through incorporation of fluorine substitutions. We also successfully developed a new amidation reaction for the preparation of peptide-aminoaryl PM conjugates using selenocarboxylate and azides, thus avoiding the use of unstable basic nucleophilic amine intermediates.



Results: The prodrugs were shown to be stable under physiological conditions including whole blood. The nitroaryl phosphoramides were shown to be excellent substrates of *E. coli* nitroreductase and highly cytotoxic towards nitroreductase-expressing V79 and SKOV3 cells. When the peptide used is a substrate of prostate-specific antigen (PSA), the peptide-aminoaryl PM conjugates were activated by PSA and have been shown to be selectively more cytotoxic to PSA-producing LNCaP prostate cancer cells than to DU145 cells that do not express PSA.
Conclusions: These prodrugs could potentially be developed into clinically useful chemotherapeutic agents for the targeted treatment of cancer.
Authors' disclosure statement: A world-wide patent application (WO 2008/067495) has been filed to cover the targeted prodrugs and the novel synthetic process.

The Impact of Inflammatory Responses on Taste Bud Cell Turnover and Function.

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Background: Taste disorders impact negatively on general health and quality of life. Although many conditions can contribute to or exacerbate taste deficits, viral and bacterial infections are among the most common causes. The underlying molecular and cellular mechanisms, however, are largely unknown. We hypothesize that inflammatory agents, particularly interferons (IFNs), may be involved in the infection-induced taste disorders.
Methods: Quantitative real-time polymerase chain reactions (PCR), in situ hybridization and immunohistochemistry were used to determine the presence of IFN signaling pathways in taste buds. Primary tissue cultures of taste bud-containing lingual epithelia were stimulated with recombinant IFNs to assay the activation of these IFN pathways. Animal models mimicking the viral and bacterial infections were established to assess the impact of infection on gene expression in taste buds. Finally, the effect of systemic administration of IFNs on taste bud cell turnover was also evaluated.
Results: IFN signaling pathways, including IFN receptors and their downstream components: protein kinases JAK1 and TYK2, and transcription factors STAT1, STAT2 and IRF9 were present in subsets of taste bud cells. Incubation of recombinant IFNs with cultured taste tissues activated the IFN signaling pathways, leading to the phosphorylation of the transcription factors. Intraperitoneal injection of lipopolysaccharide or polyinosinic:polycytidylic acid up-regulated the expression of IFN-inducible genes in taste papillae whereas the systemic administration of recombinant IFNs resulted in the increased apoptosis in the taste buds.
Conclusions: These findings suggest that bacterial and viral infection-induced IFNs can act directly on taste bud cells, affecting their cellular function in taste transduciton, and that IFN-induced apoptosis in taste buds may cause abnormal cell turnover and skew the representation of different taste bud cell types, which eventually lead to the development of taste disorders.

Combinational therapeutics Targeting Laboratory and Clinical Isolates of HIV-1

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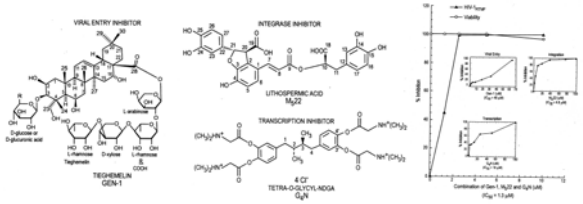
Background: Most if not all viruses, including replication-competent mutants, are host dependent. They require the participation of certain cellular factors to sustain viral growth. Cellular factors, unlike viral proteins, are not under mutational pressure and are generally structurally invariable. Some of the viral motifs interacting with these cellular factors also remain evolutionarily stable. Thus, inhibitors that block the usage of these conserved factors at different stages of vial life cycle are likely to be good candidates for mutation-insensitive antiviral drugs.
Methods: A new cocktail of small organic molecules, targeted specifically at the highly conserved motifs of virus/host interactions at different steps of the viral life cycle (viral entry, integration, and pro-viral transcriptions) was developed. Using specific bioassay-guided purification methods, including counter current chromatography, Waters Co's alliance HPLC 2695 separations module equipped with photodiode array detector, and empower chromatogradify manager, several classes of therapeutically important molecules have been isolated from plants and chemically identified.
Results: Structures of three anti-HIV inhibitors, GEN-1, $M_5$22, and G_4N targeting viral entry, integration, and pro-viral transcription respectively, are shown in Figure 1. When tested alone, GEN-1 has an IC_{50} of 40 μM, $N_5$22 an IC_{50} of 4.6 μM, and G_4N an IC_{50} of 18 μM. When tested in combination, they can block HIV-1 production in culture CD4+ cells synergistically against a variety of HIV strains at an IC_{50} of 1.3 μM. They are equally active in targeting mutant strains that are highly resistant to currently available drugs against HIV protease and reverse transcriptase as compared to the wild type viruses. In comparison, cytotoxicity for the three drugs combined in H9 cells was also analyzed using MTT assay. As further shown in Figure 2, cellular toxicity to H9 cells with combined was not detected in the presence of the three drugs, when 12 μM of each was used.



Application of Electrochemical Detection to the Determination of (A) Trace Amounts Minoxidil in Hamster Ear Skin Follicles (Magic Bullet-like Delivery) and (B) Residual Hydrogen Peroxide Present in the Minoxidil Formulation Excipients

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Background: A highly sensitive electrochemical method was needed for the determining (A), the low concentrations of the hair growth drug, Minoxidil, within hamster ear hair follicles (Magic Bullet-like Delivery), as well as, (B), the low levels of hydrogen peroxide in the formulation excipients used in the Topical Minoxidil formulations.
Methods: The Minoxidil located specifically in the sebaceous glands of the hamster ear was isolated from the skin and hair follicles in different skin layers by treatment with aqueous trichloroacetic acid followed by acetonitrile. The amount of Minoxidil in the drug containing supernatant layer was determined by liquid chromatography with detection by electrochemical oxidation (LCEC). Quantitation of the low residual levels of hydrogen peroxide in the formulation excipients was also achieved with LCEC by oxidation using the platinum electrode or by reduction using a wired enzyme electrode with dual channel electrochemical detection.
Results: The lower detection limit for Minoxidil was found to be 1 ng/ml using LCEC. The analytical recoveries ranged between 94.4-103.1% and linearity was excellent up to 250 µg/ml with a regression coefficient (r^2) of 0.9988. The LCEC method is more cost effective and the detection limit is similar to that of the widely used radiolabeled scintillation method. The analysis time for determination of the residual hydrogen peroxide present in the formulation excipients was 1 min and the detection limit was 10 ng/mL using the platinum electrode, whereas, the detection limit was 1 ng/mL using the wired enzyme electrode. Peak purity was assured by showing that the peak ratios were constant at different potentials on the dual electrodes.
Conclusions: The LCEC method allows rapid determination of the Magic Bullet-like delivery of Minoxidil to the hair follicles with levels as low 1 ng/mL, as well as, the hydrogen peroxide levels as low as 1 ng/mL in various excipients in the Minoxidil Formulations.

Drug-Peptide Conjugates with Antitumour or Anti Parasite Activity

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Background: Peptide/protein carriers can be used as soluble drug conjugates for "passive targeting" resulting in altered pharmacokinetics (e.g. accumulation in tumour), increased intracellular uptake, decreased non–specific toxicity and immunogenicity.
Methods: We have developed a new group of water-soluble oligo- or polypeptid-drug-conjugates in which daunomycin (Dau), methotrexate (MTX) or other anitumour agents are coupled to amphoteric or polycationic branched polypeptides or to oligoarginine. Toxicity, *in vitro* and *in vivo* antitumor activity of conjugates with daunomycin were investigated using sensitive and multidrug resistant, mouse (L1210 and P388 leukemia, S180 sarcoma, MXT breast carcinoma) and human (HL60 leukaemia) tumors. The antiparasitic activity of the MTX containing conjugates was analysed also *in vitro* and *in vivo* using *Leishmania donovani* infected macrophages and animals.
Results: We found that attachment of peptide to the bioactive cargo significantly improved the antitumour or antiparasitic properties of the drug, respectively and also significant reduction of drug related side effects could be demonstrated.
Conclusions: The covalent conjugation of drugs with antitumour or antiparasite activity to oligoarginine or macromolecular polypeptide typa carrier could be useful strategy to develop new compounds with improved therapeutic efficacy.

Characterised Nanomaterials for Biomarker Profiling Based Cancer Detection

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Background: Proteomics pattern analysis based biomarker discovery has recently emerged as a novel tool for early diagnostics of diseases including various cancers. In this presentation, the development of derivatized nano-structured affinity materials such as carbon nano-tubes, nano crystalline diamond, fullerene and carbon nanofiber derivatives etc. for efficient material enhanced laser desorption ionization time of flight mass spectrometry (MELDI-TOF MS) based protein profiling and biomarker detection is reported (1).
Methods: The utilization of nano-structured affinity materials with various surface properties (hydrophilic, hydrophobic, etc.) enabled rapid and reliable protein profiling to obtain characteristic mass fingerprints for rapid and reliable biomarker discovery. The applicability of MELDI-TOF-MS for rapid protein profiling was evaluated with human serum samples.
Results: First, derivatised carbon nano-tubes (2) were explored to reveal protein profile differences in human body fluids e.g., serum, by MELDI-TOF-MS (Figure 1) that can be related to disease and subsequently find relevant biomarkers.

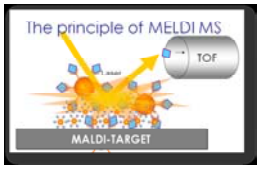

Figure 1. Principle of material enhanced laser desoprtion ionisation mass spectrometry (MELDI-MS)

The versatility of this technology is meant to increase the amount of information from biological samples at the protein level, which will have a major impact to serve the cause of diagnostic markers. The unique properties of carbon nano-tubes were exploited for manipulating this nano-structured material to construct a novel tool for efficient protein profiling and biomarker discovery by MELDI-TOF-MS. Another nanotechnology approach utilized attachment of specific parts of biomolecules to nano-crystalline diamond surface that has been selectively prepared to contain hydrophobic and hydrophilic regions (3). Nano-crystalline diamond surfaces were selected because of their unique characteristic of enormous bio-compatibility and chemical inertness, i.e. lack of cross-reactivity with such analytes as serum peptides and proteins. Finally, derivatisation of fullerenes (4, 5) and carbon nano-fibers (6) were found to alter its adsorption characteristics toward proteins, thus differences in protein profiling mass patterns were expected when employing the fullerene derivatives as MELDI carrier materials. The success of individual derivatisation steps of these and other materials surface as well as physical properties, e.g. surface area, porosity were measured by a novel near-infrared spectroscopic (NIRS) method, enabling an extreme fast and presice physico-chemical characterization (7-9).
Conclusions: The novel technology allows to distinguish cancer, non-cancer, benign in case of prostate cancer with a sensitivity and selectivity > 85%.

1) M. Najam-ul-Haq, M. Rainer, Z. Szabó, R. Vallant, C. W. Huck*, G. K. Bonn, J. Biochem. Biophys. Meth., (2007), 70, 319-328 **2)** M. Najam-ul- Haq , M. Rainer, T. Schwarzenauer, C.W. Huck* , G.K. Bonn , Anal. Chim. Acta, 561, 2006, 32-39 **3)** M. Najam-ul-Haq, M. Rainer, C.W. Huck, G. Stecher, I. Feuerstein, D. Steinmüller, G.K. Bonn, Current Nanoscience, 2006, 2, 1-7 **4)** R.M. Vallant, Z. Szabo, S. Bachmann, R. Bakry, M. Najam-Ul-Haq, M. Rainer, N. Heigl, C. Petter, C.W. Huck, G.K. Bonn, Anal. Chem., 2007, 79(21), 8144-8153 **5)** M. Najam-ul-Haq, M. Rainer, N. Heigl, Z. Szabo, R. Vallant, C.W. Huck*, H. Engelhardt, R. Bischoff, G.K. Bonn, Amino Acids, 2008, 34, 279-286, DOI 10.1007/s00726-007-0492-5 **6)** A. Greiderer, M. Rainer, M. Najam-ul-Haq, R.M. Vallant, C.W. Huck*, Günther K. Bonn, , Amino Acids, 2008, in press **7)** N. Heigl, C.H. Petter, M. Najam-Ul-Haq, M. Rainer, R.M. Vallant, G.K. Bonn, C.W. Huck*, J. Near Infrared Spectrosc., 2008, in press **8)** C.W. Huck*, R. Ohmacht, Z. Szabo, G.K. Bonn, J. Near Infrared Spectrosc., 14, 2006, 51-57 **9)** R.M. Vallant, Z. Szabo, L. Trojer, M. Najam-Ul Haq, M. Rainer, C.W. Huck, R. Bakry, G.K. Bonn, J. Proteom. Res., (2007), 6(1), 44-53.

Glucose transporters in the blood-brain barrier

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Backround: The central nervous system and peripheral nerves are guarded against free access from the outside by the blood-brain, blood-cerebrospinal fluid and blood-nerve barriers. The glucose transporter GLUT1 mediates the specific transfer of glucose across these barriers while GLUT3 is a high-affinity isoform of Type I glucose transporter expressed mostly in neurons where it is believed to be the main glucose transporter isoform. As for a long time it was an open question whether GLUT1 and GLUT3 are present in the olfactory system the aim of the present study was to give answers to these questions.
Methods: In the study including 20 male Wistar rats (4 weeks old) mucous membranes of the olfactory region were studied by double immunofluorescence labeling. Rabbit anti-GLUT1, guinea pig anti-GLUT1, rabbit anti-chicken tubulin, rabbit anti-GLUT3 and mouse anti-PGP served as primary antibodies.Fluorescein isothiocyanate-labeled donkey antiguinea pig immunoglobulin G (IgG), dichlorotriazinyl amino fluorescein-labeled and rhodamine red X-labeled donkey anti-rabbit IgG, and Cy3-labeled donkey anti-mouse IgG were used as secondary antibodies.
Results: The studies indicated the abundant presence of GLUT1 in the endothelial cells of olfactory mucosa while the upper cells of olfactory epithelium *(cellulae neurosensoria olfactoriae)* stained srongly positive for GLUT3. Anti-tubulin antibody strongly stained the apices of the olfactory epithelial cells as well as nerve fiber bundles emanating from the epithelium. Anti-PGP antibody stained olfactory receptor neurons in the olfactory epithelium and the nerve fibers running underneath .
Conclusions: The immunolocalization of GLUT1 in the endothelial cells of olfactory mucosa and GLUT3 expressed primarily in olfactory receptor neurons allow glucose to cross the blood-brain barrier and enter neurons.
Moreover, the results also showed that PGP serves as a marker for the olfactory epithelium (nerve fibers emanating thereof) and that tubulin acts as a marker for the nerve fibers in olfactory mucosa.

Genetic Variant of *KIF6* Predicts both Increased Risk for Coronary Events and Greater Benefit from Statin Therapy: an Overview of Genetic Studies of the CARE, WOSCOPS, and PROVE IT - TIMI 22 Trials

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PACKARD CJ[3], SACKS FM[2,4], SHEPHERD J[3], DEVLIN JJ[1] AND BRAUNWALD E[2]

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Background: Statins are the drugs of choice for primary and secondary prevention of coronary heart disease; however, response to statin therapy varies dramatically between individuals. Aim: To identify and validate genetic polymorphisms that are associated with risk of coronary events and differential response to statin therapy.
Methods: We used Cox proportional hazards models that adjusted for traditional risk factors to investigate the effect of pravastatin therapy versus placebo by *KIF6* 719Arg carrier status and the association between *KIF6* 719Arg carrier status and coronary events in the CARE and WOSCOPS trials and to investigate the effect of high-dose atorvastatin therapy versus standard-dose pravastatin therapy in the PROVE IT–TIMI 22 trial.
Results: The 719Arg variant of Trp719Arg (rs20455), a polymorphism in kinesin-like protein 6, was associated with greater risk of coronary events and greater benefit from pravastatin versus placebo. In placebo–treated patients, carriers of the *KIF6* 719Arg allele (59% of CARE and WOSCOPS) had a hazard ratio of 1.50 (95% CI 1.05 - 2.15) in CARE and an odds ratio of 1.55 (95% CI 1.14 - 2.09) in WOSCOPS. Among 719Arg carriers, the absolute risk reduction by pravastatin was 4.89% (95% CI 1.81 - 7.97) in CARE and 5.49% (95% CI 3.52 - 7.46) in WOSCOPS. In contrast, no significant risk reduction was observed among noncarriers. In PROVE IT–TIMI22, benefit from high-dose, compared with standard-dose, statin therapy was significantly greater in the 59% of the cohort who were carriers (hazard ratio 0.59, 95% CI 0.45 - 0.77) than in noncarriers (hazard ratio 0.94, 95% CI 0.70 - 1.27); p=0.018 for interaction between 719Arg carrier status and treatment. Absolute risk reduction was 10.0% in carriers versus 0.8% in noncarriers.
Conclusions: 1) Carriers of the *KIF6* 719Arg allele are at increased risk of coronary events, and pravastatin therapy substantially reduces that risk. 2) Carriers of 719Arg receive significantly greater benefit from high-dose statin therapy than do noncarriers. 3) In all three trials, noncarriers of 719Arg (representing over 40% of the populations) did not benefit from statin therapy. Since statin therapy may obscure benefits of other cardiovascular drugs, demonstrating the benefit of new cardiovascular compounds over highly potent statins may be more successful in statin non-responders identified by the *KIF6* polymorphism.

Peroral Colon-Specific Delivery of Insulin Based on Novel Acrylic-Terpolymer Microcapsular Devices

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Background: Recently, colon has attracted much attention as a potentially delivery site for perorally administered peptide-based drugs. In this context, we have developed novel delayed-release microcapsules (MCs) with a membrane of newly synthesized acrylic terpolymers as a prototype of colon-specific delivery device for peptide-based drugs. Aims: 1) To prepare insulin-containing MCs. 2) To evaluate stability, in vitro release and in vivo absorption behavior of the microencapsulated insulin.
Methods: An aqueous colloidal dispersion of terpoly(ethyl acrylate/methyl methacrylate/2-hydroxyethyl methacrylate) with molar ratio of 95:85:40 was synthesized by emulsion polymerization. The MCs composed of a lactose core (90–105 microns), a layer of bovine pancreatic insulin with a protease inhibitor (bacitracin) and an absorption enhancer (sodium glycocholate), and a release-delaying coat of the terpolymers were prepared by the air-suspension spray coating process. The obtained MCs were heat-cured at 40˚C for 6h and then subjected to stability assay by an HPLC method, release test using a paddle method and absorption study of insulin after peroral administration to gastro-physiology-regulated beagle dogs.
Results: The MCs with mass median diameters of 175–226 microns were obtained at the yield of 86–93%. Degradation of insulin during the spray-coating process and post-thermal curing was only few % when the process temperatures were set to below 40˚C. The heat-cured MCs showed delayed-release of insulin in a pH-independent manner. The lag-time of drug-release could be controlled by altering the coat thickness of terpolymers. Peroral administration of the MCs with 6-h lag-time to the beagle dogs revealed a significant reducing effect of blood glucose level (the pharmacological abailability was estimated to be 5.1%) while that of the MCs with 3-h lag-time did not. Moreover, the microencapsulated insulin was found to be stable and its release profile was not changed significantly even after the storage at 4˚C for 2 years.

Conclusions: 1) Microencapsulation of insulin with the terpolymers was possible without significant degradation of insulin. 2) The microencapsulated insulin was stable for 2 years, released pH-independently in a delayed mannar, and effective to enhance in vivo insulin absorption thorugh the colon.

Oral Cancer: Molecular Pathogenesis and Novel Therapeutic Approach

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Background: Aberration of signal transducers in PI3K/AKT pathway has been found in many human cancers including oral cancer and may play a critical role in carcinogenesis of those cancers. Advanced research on the treatments of oral cancer using novel agents targeting on PI3K/AKT signaling pathway are investigated in many laboratories with promising results. The objectives of the present study were (1) to investigate protein expression of pan AKT and its phosphorylated form, p-AKT, in oral squamous cell carcinoma (OSCC) tissues of 20 Thai patients, (2) to analyze mRNA expression of three isoforms of AKT; AKT-1, -2, and -3 and protein expression of pan AKT, AKT-1, and AKT-2 in OSCC cell lines and human oral keratinocytes (HOK), and (3) to analyze protein expression of vimentin and E-cadherin in OSCC and HOK cell lines.
Methods: The expression of pan AKT and p-AKT in OSCC tissues was studied by immunohistochemistry. The mRNA expression of AKT-1, -2, and -3 in OSCC cell lines and HOK was analyzed by RT-PCR and the protein expression of pan AKT, AKT-1, AKT-2, vimentin, and E-cadherin was studied by Western blot assay.
Results: The results showed that pan AKT and p-AKT were overexpressed in 95% and 100% of OSCC cases, respectively. We observed more intense expression of pan AKT and p-AKT at the invasive fronts of some OSCC tissues. Pan AKT protein was also overexpressed in all OSCC cell lines in comparison with HOK. Interestingly, AKT-1 and -2 mRNA of OSCC cell lines were only constitutively expressed in comparison with HOK. AKT-3 mRNA appeared to be minimally expressed in OSCC cell lines and HOK. The Western blot analysis revealed that AKT-2 but not AKT-1 was overexpressed. Additionally, vimentin was upregulated while E-cadherin was downregulated.
Conclusions: These findings suggested that overexpression of pan AKT particularly AKT-2 and p-AKT may be involved with OSCC carcinogenesis and post-transcriptional modification of the expression of AKT isoforms in OSCC may occur. In addition, OSCC cells may undergo epithelial-mesenchymal transition since their epithelial maker (E-cadherin) was reduced whereas their mesenchymal marker (vimentin) was increased.

Antiplasmodial and Immunomodulating Activity of Some Sudanese Herbal Medicine with emphasis on Pristimerin as Antiplasmodial and Antileishmanial Agent

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Background: The Sudan is being the largest country in Africa, covering an area of one million square miles with the different metrological and polyethnic, with a diverse flora. Most people in rural areas rely on traditional medicine for the treatment of many infectious diseases.
Objectives: WHO has recently advocated the use of traditional medicine where appropriate health services become inaccessible, therefore, the study aims to investigate the potential antiplsmodial, antleishmanial activity of some medicinal plants and to detect their effect on human lymphocytes proliferation which may imply the ability to potentiate the human immune system.
Material and methods: Forty-nine plant parts representing 26 species from 15 families were extracted and screened for their activity on chloroquine sensitive strains 3D7 and Dd2. Plants were collected according to their traditional use and / or to their taxonomical affiliation to their families that had been reported to have antimalarial activity.
Results: Thirty-four methanol extracts (59%) exhibited significant activity against 3D7 with IC_{50} values ≤ 50 µg/ml, while twenty-one extract (57%) showed antiplasmodial activity on Dd2 with IC_{50} values ≤ 50 µg/ml. On the other hand, thirteen extracts (22%) and ten extracts (18%) only showed an activity with IC_{50} values ≤ 5 µg/ml on 3D7 and Dd2; respectively. Human lymphocytes treated with the most of extracts demonstrated a minimum level of toxic inhibitory effect at concentration ≥ 100µg, whereas *Sonchous cornatus*, *Balanites aegyptiaca*, *Acacia nilotica* and *Tamarindus indica* enhanced lymphocytes proliferation. Bioactivity directed fractionation of the chloroform extract of the root bark of *Maytenus senegalensis* resulted in the isolation and characterization of the quinonemethide triterpene, (20α)-3-hydroxy-2-oxo-24-nor-friedela-1(10),3,5,7-tetraen-carboxylic acid - (29)- methylester (pristimerin). The structure was elucidated by spectroscopic techniques. The *in vitro* antiplasmodial activity of the isolated compound against chloroquine-resistant strain (Dd2) of *Plasmodium falciparum* was IC50 = 0.5µg/ml and its *in vitro* antileishmanial activity performed on promostigotes of *Leishmania major* was IC50 = 6.8 ± 0.8 µg/ml while the cytotoxicity on lymphocyte proliferation model was detected at IC50= 6.8 ± 0.8 µg/ml.
Conclusion: The promising response of *Acacia nilotica* and *Mayenus senegalensis* conclude that some Sudanese plants used in traditional medicine possess a potent antimalarial activity with minor effects on lymphocytes proliferation. These plants have been subjected to long-term clinical trials in folk medicine and hence we propose that these plants should be further investigated.

Can Erythropoietin be used to prevent brain injury in African Children with Cerebral Malaria?

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Background: Cerebral malaria is associated with high mortality and long-term neuro-cognitive deficits. Erythropoietin has shown neuro-protective properties in different neurological disorders. We hypothesized that the outcome of cerebral malaria is modified by the responses of this cytokine to hypoxia and that high plasma and cerebrospinal (CSF) levels would prevent neurological damage. AIM. Determine the relationship between plasma and CSF erythropoietin and the outcome of cerebral malaria in African children.
Methods:
We measured erythropoietin in paired samples of plasma and CSF of 124 children with cerebral malaria. Patients were categorized into 3: 76 without deficits, 32 with deficits and 16 who died. The median (IQR) plasma erythropoietin were compared. Logistic regression models were used to identify risk and protective factors associated with sequelae.
Results: The median (IQR) plasma concentrations of erythropoietin were 123(29-1,726)U/L, 184(23-694)U/L and 278(96-1,852)U/L in children who died, survived with and without sequelae respectively. Conditional logistic regression analysis matching the 32 patients with sequelae to 64 patients without sequelae stratified for hemoglobin level estimated that plasma erythropoietin >200 U/L was associated with greater than 80% reduction in the risk of sequelae (adjusted OR 0.18, 95%CI 0.05-0.93). Both the level of erythropoietin and the protective effect of erythropoietin were greater in younger children.
Conclusions: 1) High levels of erythropoietin were associated with reduced risk of neurological deficits. 2) The age-dependent erythropoietin response to anemia and the age-dependent protective effect may influence the clinical epidemiology of cerebral malaria. 3) These data support further study of erythropoietin as an adjuvant therapy in cerebral malaria.

Factors Affecting Toxicity and Efficacy of Injectable Nanomedicines: Significance on Particle Size

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Background: Nanomedicine is the application of nanotechnology to medicine. Recently, the National Nanotechnology Initiative referred to nanotechnology as 'the understanding and control of matter at dimensions of roughly 1 to 100 nanometers, where unique phenomena enable novel applications'. The usefulness of cancer chemotherapy is restricted by the dose-limiting toxicity of cytotoxic anticancer drugs and the occurrence of side effects. Toxicity arises from the lack of efficient selectivity of traditional anticancer drugs for malignant cells. That is, there is no decrease in the distribution of drug in normal tissue (target organ) and concomitant increase in distribution in the tumor tissue with the use of chemotherapeutic drugs. The purpose of this article is to review the passive targeting properties of several injectable water-soluble polymeric nanomedicines that are between 1 and 100 nm in size, to illustrate the impact of polymeric nanomedicines on toxicology. I consider several biodegradable polymeric nanomedicines that are between 1 and 100 nm in size, and discuss the impact of this technology on efficacy, pharmacokinetics, toxicity and targeting. In addition to above previous discussion (Igarashi, 2008, published on Toxicol. Appl. Pharmacol., 229:121-134), I discuss the lower size limit around 5 nm rather than 1 nm for the passage of intact nanomedicines.
Discussion: The degree of toxicity of polymeric nanomedicines is strongly influenced by the biological conditions of the local environment, which influence the rate of degradation or release of polymeric nanomedicines. The dissemination of polymeric nanomedicines in vivo depends on the capillary network, which can provide differential access to normal and tumor cells. The accumulation of nanomedicines in the microlymphatics depends upon retention time in the blood and extracellular compartments, as well as the type of capillary endothelium surrounding specific tissues. The toxicity or efficacy of intact nanomedicines is also dependent upon tissue type, i.e., non-endocrine or endocrine tissue, spleen, or lymphatics, as well as tumor type (Igarashi, 2008). Electron microsocopic and recent immunobiological studies of endothelial fenestral diaphragm showed that the lower size limit affecting the distribution of nanomedicines in normal tissue may depend on the size/shape of communicating channel spanned by diaphragm.

Suppression of Membrane Microvesiculation – a Possible Anticoagulant and Anti-Tumor Progression Effect of Heparin

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Background: Heparin was found a successful drug not only in preventing thromboembolic events but also in slowing down the development of some types of cancer. As microvesiculation of membranes is enhanced both in hypercoagulabile states as well as in cancer, a hypothesis of anticoagulant and anti-tumor-progression effects of heparin is put forward: heparin mediates an attractive interaction between membranes and suppresses microvesiculation by adhesion of buds to the mother membrane before they become free vesicles.

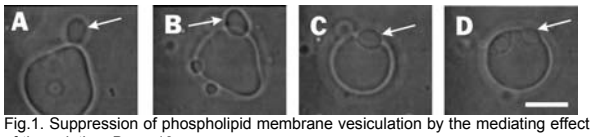

Fig.1. Suppression of phospholipid membrane vesiculation by the mediating effect of the solution. Bar = 10μm.

Methods: Giant phospholipid vesicles were created by electroformation and observed under the phase contrast microscope. Blood plasma and heparin were added to the suspension of vesicles. A consequent adhesion between vesicles was assessed by measuring the average contact angle between the adhered vesicles.
Results: Addition of 10 μl of therapeutic concentration of Fraxiparine, GlaxoSmithKline, UK, mixed and preincubated with plasma, into 55 μl of vesicle suspension caused stronger mediating effect than addition of plasma alone. The average contact angle 30 minutes after the addition of the sample was 98 degrees, while it was 86 degrees due to plasma alone, the difference being statistically significant ($p<0.001$). The same concentration of heparin without plasma did not cause adhesion of vesicles.
Conclusion: Heparin in therapeutic concentrations enhances the ability of plasma to cause adhesion between membranes and may thereby suppress microvesiculation. This represents a possible biophysical anticoagulant mechanism of heparin.

The impact of non-fucosylated therapeutic antibodies in humans *in vivo*.

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Background: Antibody-dependent cellular cytotoxicity (ADCC) has recently attracted attention as an important mechanism of the clinically effective therapeutic antibody. However, the ADCC of currently licensed fucosylated therapeutic IgG1s has been found to be strongly inhibited by plasma IgG through competition for binding of the therapeutics to FcγRIIIa on NK cells, which causes such a high dose requirement in antibody therapies. Here, we investigated whether or not ADCC of non-fucosylated therapeutic IgG1 is also influenced by plasma IgG.
Methods: *Ex vivo* ADCC upon CD20-positive human B cells was induced by incubation of human whole blood with non-fucosylated and/or fucosylated anti-CD20 IgG1s rituximab, and quantified by measuring the remaining CD19-positive human B cells using flow cytometry.
Results: Non-fucosylated anti-CD20 showed markedly higher *ex vivo* B-cell depletion activity than its fucosylated forms in the presence of plasma IgG. The efficacy of fucosylated anti-CD20 was greatly diminished in plasma, resulting in the need for a high concentration (over 1 μg/mL) to achieve saturated efficacy. In contrast, non-fucosylated anti-CD20 reached saturated ADCC at lower concentrations (0.01 to 0.1 μg/mL) with much higher efficacy than fucosylated counterparts through improved FcγRIIIa binding. Importantly, the high ADCC efficacy of non-fucosylated therapeutic antibodies has been shown to be inhibited by the fucosylated forms through the competition for binding to the antigen on target cells.
Conclusions: Our data showed that non-fucosylated IgG1, not including fucosylated forms, can evade the inhibitory effect of plasma IgG on ADCC through its high FcγRIIIa binding, and elicit saturable ADCC. Hence, the application of non-fucosylated antibodies is expected to be a promising approach as next-generation therapeutic antibodies with improved efficacy, even when administrated at low doses in humans *in vivo*. Clinical trials using non-fucosylated antibody therapeutics are currently underway.

Expression of Estrogen Receptors during Postnatal Development of the Brain

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Background: Estrogen receptors (ERs) mediate effects of estrogens, which are important for regulating neuroendocrine, physiological, and reproductive functions in matured and developing mammalian brains. The two ER subtypes, ERα and ERß, are expressed in many brain regions of rodents. However, the expression pattern is different between ERα and ERß, and the expression of each receptor can change during development. The purpose of this study is to compare the expression level and cellular distribution between the two ERs during postnatal development of the rat cerebellum.
Methods: Expression levels of ERα and ERß mRNAs were examined using quantitative real-time RT-PCR (qRT-PCR), at postnatal days 7 (P7), P14, P21, and in adulthood, in the rat cerebellum. Furthermore, distributions of mRNA and protein for both ERs in the developing cerebellum were analyzed using in situ hybridization and immunohistochemistry. Double-label immunofluorescence for ERα and ERß was utilized to determine whether the two receptors were expressed within the same cell.
Results: QRT-PCR demonstrated that levels of cerebellar ERα mRNA in neonatal pups were significantly higher than those in adults. In contrast, expression levels of cerebellar ERß mRNA remained significantly unchanged during postnatal development. In situ hybridization and immunohistochemistry demonstrated that ERα mRNA and protein were predominantly expressed by Purkinje cells. ERα-immunoreactive Purkinje cells were distributed in most lobes at P14 and P21, and some of them were co-localized with ERß. However, only a few ERα-immunoreactive cells were observed in the adult cerebellum. ERß expression occurred in Golgi type neurons in the granular layer at P7, Purkinje cells at P14, and basket cells in the molecular layer at P21, and was detected in all the cell types in the adult cerebellum.
Conclusions: 1) ERα expression was transiently increased during the time when Purkinje cell dendritic growth and synapse formation proceed, suggesting that a role for ERα in Purkinje cell differentiation. 2) The expression profile of ERß suggests a role for ERß associated with neuronal differentiation and maintenance. 3) The discrete expression profiles for ERα and ERß in the developing cerebellum suggest the two ERs play distinct roles in cerebellar development.

Improvement of PGH-Synthase and Papain Stability in Pectin Contained Systems Used for Healing of Skin Wounds

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Background: Prostaglandin H synthase (PGHS, EC 1.14.99.1) mediates a variety of physiological processes. Papain (EC 3.4.22.2) is protease that is used as debridement agent. The goal of the present study was to obtain materials applicable for treatment of skin wounds by means of these enzymes immobilization in biopolymer pectin.
Methods: PGHS was isolated from bovine vesicular glands. Both enzymes were immobilized on pectin films and tested for storage stability. Papain was also prepared in spray with 6% pectin gel to form a thick film on a skin. The papain stability in aerosol spray was defined at 65°C under pressure of air and N_2 (up to 90 psi) as well as during its storage.
The effectiveness of immobilized PGHS and papain on tissue repair was tested in mice and rabbits, respectively, by measuring linear size of wound. Prostaglandin E_2 (PGE_2) was measured by HPLC. Moreover, applications of pectin/papain films on wounds of voluntary patients were performed.
Results: Immobilized PGHS retained 41 and 12% after 60 days of storage at -15º and 4ºC, respectively, while in solution it was inactivated to 14% after 24 h. Papain retained 98% of activity on pectin film and only 8% in spray after 6 months of its storage at 4ºC, while in solution it was inactivated after 1 week. The papain activity and stability were decreased under high propellant pressure in solution and were increased in pectin gel.
Application of immobilized enzymes accelerated animal wound healing. The PGE_2 level and specific rate of healing progression, calculated as a tangent of lines obtained in semi-logarithmic coordinate, were increased under PGHS treatment. A kinetic scheme was proposed to describe the effect of PGHS.
Wound healing of treated patients was accelerated without any negative secondary effects.
Conclusions:
1. Immobilization on pectin contained systems allows for enzymes stabilization and simplifies their application on skin, it may be considered for other drugs.
2. Prostaglandins level regulation, for example by PGHS addition, is a mechanism to influence skin wound repair.
3. Kinetics of the healing process of a linear surgical wound may be described by the logistic curve that led to kinetic model describing the effect of PGHS on wound repair process.

Molecular investigation of drug resistant *Neisseria gonorrhoeae* clinical isolates

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Background: The introduction of new adequate methods for correct species identification and susceptibility testing of *N. gonorrhoeae* (*NG*) remains an actual problem of sexual transmitted diseases monitoring. Aims: 1) to show the suitability of the MALDI Biotyper, a system based on mass spectrometry (MS) profiling, for *NG* species identification, 2) to determine known genetic markers of penicillin (PEN), tetracycline (TET) and fluoroquinolones (FQ) resistance in gonococci, 3) to study the relation between genetic markers of drug resistance and the susceptibility profile of clinical *NG* strains.
Methods: This study included *NG* clinical isolates collected from different regions of Russia. Susceptibility testing to PEN, TET and FQ was performed by the agar dilution method according to CLSI. Single colonies of fresh bacterial cultures were tested by the MALDI Biotyper system (www.bruker.com) using a Microflex LT MALDI-TOF mass spectrometer. Total genomic DNA from *NG* strains was isolated using the “DNA express” kit (Lytech Ltd, Russia). The presence of bla and tet(M) genes was analyzed by PCR. Mutations in *NG* genes (rpsJ, por1, penA, ponA, gyrA, parC, mtrR, norM) associated with drug resistance were detected by primer extension reaction followed by MALDI-TOF MS analysis or by sequencing.
Results: Totally, 293 bacterial isolates previously identified as *NG* were investigated. Susceptibility levels to PEN, TET and FQ were found to be 26 %, 36 %, and 54 %, respectively. For 280 samples the MS profiles were matched to strain *NG* ATCC 49226. The other MS profiles (n=13) were similar to each other but rather different from *NG* as well as from *N. meningitidis*, non-pathogenic *Neisseria* and further 1671 different microorganisms stored in the MALDI Biotyper library. Further 16S RNA sequencing referred them as unknown species from the genus *Ralstonia*. The distribution of genetic drug resistance markers was studied for certain susceptibility groups of *NG* strains. Their positive predictive value was found to be different for FQ (90 %), PEN (91 %) and TET (82 %).
Conclusions: 1) The MALDI Biotyper system is a highly suitable tool for the correct *NG* species identification, 2) the surveillance of genetic markers may be useful for *NG* monitoring.

Organ protective effects of tunicamycin as an endoplasmic reticulum stress inducer

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Background: Various disturbances cause the endoplasmic reticulum (ER) dysfunction and thereby induce the accumulation of unfolded proteins in the ER, in turn leading to “ER stress”. ER stress induces unfolded protein response (UPR), which is aimed at ameliorating the cell stress but triggers apoptosis when ER stress is excessive and/or prolonged. Tunicamycin (TUN) is an ER stress chemical inducer and has renal toxicity by causing ER stress-induced apoptosis. Here we hypothesized that a low dose of TUN, which induces an adaptive UPR rather than proapoptotic UPR, is protective against ER stress-related kidney diseases. Thus, we investigated if therapeutic approach targeting ER stress is effective in a model of glomerulonephritis (GN).
Methods & Results: ER stress status was assessed in GN rats by detection of UPR: 1) expression of ER stress-inducible chaperones, 2) intracellular signaling for shut down of translation. By immunohistochemistry and Western blot analysis, UPR was significantly increased in damaged glomeruli of GN rats and it was associated with the disease manifestations. We then assessed if preconditioning of ER stress prevents the disease progression. TUN at non-nephritogenic dose (0.3mg/kg) was injected into the rats 4 days before GN induction for ER stress preconditioning. TUN pretreatment barely increased UPR in glomeruli of normal rats without affecting glomerular morphology and renal function. Interestingly, our histological analyses showed that disease progression of GN was dramatically improved by TUN ($p<0.05$). These histological improvements were associated with amelioration of proteinuria (39.4 ± 10.5 v.s. 126.1 ± 18.1 mg/day; $P<0.01$). Of note, the protective effect of TUN on the glomerular damage was associated with the modulation of UPR: excessive increase in ER stress-inducible chaperon expression and the signaling for translation shut down observed in glomeruli of GN was reduced by TUN, demonstrating that amelioration of excessive ER stress by preconditioning may contribute to improvement of glomerular injury.
Conclusions: Glomerulonephropathy is associated with excessive ER stress. ER stress preconditioning with non-nephritogenic dose of TUN ameliorates manifestations of glomerulonephropathy, suggesting the possibility of therapeutic approach targeting ER stress in the kidney.
(Grant, JSPS-19590939 to RI and -19390228 to MN)

Pathophysiological Approach against Resistant Bacteria-causing Infectious Diseases; - from Autopsy Findings to Clinical Applications –

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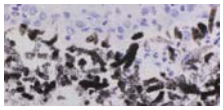
Background: Autopsy is a powerful tool for analyzing the cause of a patient's death, progression of the disease, and the therapeutic response. However, it is difficult to identify the bacterial characteristics using morphological analysis only, especially in cases of resistant-bacterial strains.
Methods: To elucidate the pathogenesis of resistant bacteria-causative infectious diseases, intrapulmonary sputum was harvested by directly inserting a swab into a resected lung at autopsy, and the bacterial composition was analyzed using both pathological and microbiological techniques from 15 patients with hematological malignancies, and the results were compared with those from 25 patients with other medical and surgical diseases. Then, we established an *in vivo* infection model to investigate kinetics of pathogens or effects of antimicrobials on slide sections by using autoradiography methods.
Results: Among the 54 bacteria strains isolated from the 40 patients, multi-drug resistant strains were significantly more prevalent in hematological group than in other diseases (16/21 vs. 11/33, p=0.002). *E. faecium* was preferentially isolated from the hematological patients, whereas the methicillin-resistant *S.aureus* was predominantly found in the non-hematological group. Even coagulase-negative *S. epidermidis* strains in hematological diseases may be diagnosed as causative bacteria of pneumonia by both bacterial and pathological techniques.
In mice or rats infected bacteria or fungus, the administration of ^{3}H-labeled cell wall substrates brought about accumulation of the compounds in the microorganisms with very low backgrounds on the pathological sections (Rt-figure represents that the deposition of RI compounds can visualize *Aspergillus* on slide sections using both pathological and autoradiography techniques).



Conclusions: Traditional pathological approach such as autopsy would extend a further analytical tool of resistant-bacteria causing infectious diseases in combination with microbiological or radiological techniques. Our new animal model could also contribute to develop new antimicrobials as well as clinical applications.

»Carbon bullet«: Fullerenol $C_{60}(OH)_{24}$ as organo-protector against doxorubicin-induced toxicity

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Background: Fullerenes are a type of carbon molecule first discovered in the mid-1980s. The most well-known of these is the buckyball, which is a closed spherical molecule, shaped like a soccer ball. Many aggressive studies are presently in progress to search for pharmaceutical products that can capitalize on the excellent bioactivity of fullerenes and their derivatives. Thus far, different fullerene properties have been put to use, such as its ability to eliminate radicals as well as its particular molecular structure. Studies have found it to be efficient in the suppression of metastases, the treatment of cerebral conditions such as Alzheimer's and Parkinson's diseases, type-C hepatitis therapy, and HIV treatment. A polyhydroxilated derivative of fullerene, named fullerenols $C_{60}(OH)_n$ is being extensively studied due to its great potential as an antioxidant. It is proposed that fullerenols may act as a free radical scavenger in biological systems, in xenobiotics, as well as radioactive irradiation-induced oxidative stress. It has demonstrated protective effects against cytotoxicity of doxorubicin (Dox) in animal models, especially by fullerenol $C_{60}(OH)_{24}$.
Methods: Sprague Dawley outbred rats with chemically induced mammary carcinomas were used to investigate a potential protective role of fullerenol against cardio-, hepato-, nephro- and pulmo-toxicity induced by Dox. According to preliminary studies on healthy adult Wistar rats our research group confirmed that 100 mg/kg (i.p.) of fullerenol administered 30 min before Dox has a protective influence on heart and liver tissue. Therefore that dose was chosen as effective against acute Dox-induced toxicity in further examinations.
Results: In our recently published paper an *in vivo – in vitro* study was examined to confirm the potential protective role of fullerenol $C_{60}(OH)_{24}$ on Dox-induced liver toxicity. The *in vivo* results (Sprague-Dawley rats) showed that treatment with Dox alone caused significant changes in the serum levels of ALT, AST, LDH and α-HBDH, as well as in the levels of MDA, GSH, GSH-Px, TAS, GR, CAT, and SOD in the liver tissue. These effects were drastically reduced for all investigated parameters by pre-treatment with fullerenol, although not for the MDA and GSH level. On the other hand, the human hepatocellular carcinoma (HepG2) cell line was continuously treated with fullerenol for 12, 24, 48 and 96 h, at concentrations of 10 and 44 μg/mL. With the aim of evaluating the modulating activity of fullerenol on Dox-induced hepatotoxicity, the cell line was concurrently treated with Dox (1 μM; 5 μM) and fullerenol (10 μg/mL; 44μg/mL) in different combinations. When the cells are treated with 5 μM Dox along with the fullerenol, a significant development of cell capability during the entire timeline can be seen. It was concluded that fullerenol has cytotoxic effects on HepG2 by itself, but when the oxidative stress is too high, the cytotoxic effects of fullerenol are overcome by its protective role as a strong antioxidant compound.
Damage to the heart muscle after Dox administration was also confirmed by changes in the ultra-structural pathology results and SOD, MDA, CAT, GSSG, GR, and TAS levels, as was a potential cardioprotective influence of fullerenol as a pretreatment agent for Dox therapy in the acute phase. Fullerenol itself, in a dose of 100 mg/kg, did not affect heart injury in rats with breast cancer. The presented results suggested that fullerenol might be a potential cardioprotector in Dox-treated individuals.
Conclusions: The key benefit of fullerenol, in contrast to other known antioxidants, is its dual function as radio-protector and organo-protector during the anticancer therapy (radio- and chemo-). However, there is a need to carry out further studies, including a chronic investigation in animals (this study was done and will be also presented) and human trials.

Therapeutic Control Chart As A Tool To Aid Drug Monitoring. A Comparison Between The Acenocoumarol And Digoxin Laboratory Control.

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Background: Retrospective data of patients under oral anticoagulant (OA) and digoxin therapy were used to estimate a reference change using a statistical method. The cumulative probability allow us to obtain it for two consecutive measurements with $p \leq 0.05$ preparing a therapeutic control chart (TCC). The present study was conducted to evaluate the statistical and clinical differences between the acenocoumarol and digoxin TCCs.
Methods: We compared two populations of patients under acenocoumarol anticoagulant (group 1) and digoxin (group 2) evaluated in our laboratory. It was calculated for each group a mean (X) and control limits: X+/-1σ and X+/-2σ (mean+/-standard deviation). For group 1 (N=323), forty-five patients without major (OA) therapy complications, with more international normalized ratio (INR) determinations in the therapeutic range (INR= 2-3) and with a normal distribution of INR values according to the Kurtosis and asymmetric coefficient, were selected. For group 2 (N=282), eighty-nine patients were considered with digossin results with normal distribution comprised from 0.8 to 2 ng/mL and borderline values.
Results: For acenocoumarol study (group 1) we found that the cumulative probability at X+/-1σ INR= 1.8 and at INR= 3 was p= 0.15 and p= 0.17, respectively. The (group 1) TCC has suggested that for results close to the therapeutic control limits, we needed at least two consecutive INR results to detect a significant over or under-anticoagulation (i.e. p 0.17x0.17= 0.02). For digoxin study (group 2), we observed that at therapeutic limit 0.8 ng/mL correspond a p= 0.27 instead at control limit equal to 2 ng/mL the probability became p= 0.04. This means that, at level of upper therapeutic limit is necessary only one measurement of digossin testing to define a risk of over administration.
Conclusions: 1) The therapeutic control chart with different cumulative probability could added analytical and clinical data to drug monitoring. The differences between the acenocoumarol and digoxin TCC could be explained through the different pharmacokinetic mechanisms. 2) The acenocoumarol TCC, requiring two control tests at level of therapeutic limits, has demonstrated the more complexity of anticoagulant therapy versus the digoxin therapy.

Reduction Of Surgical Site Infections During Paediatric Cardiac Surgery

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Background: Surgical site infections (SSIs) are a substantial cause of morbidity, mortality, and of increased costs among hospitalized patients. Patients who develop SSIs are up to 60% more likely to spend time in the intensive care unit, 5 times more likely to be readmitted to the hospital and two times more likely to die than are patients without SSIs. (1) The objective of antimicrobial prophylaxis is to reach serum and tissue drug levels that exceed, throughout surgery, the MICs for the organisms most likely encountered. Timing of antibiotic administration is essential in achieving this goal. In order to achieve low SSIs rates the antimicrobial should be administered as near to the incision time as possible and within 60 minutes of incision. (2) The primary aim of our study was to identify our current antibiotic timing administration in pediatric cardiac surgical patients and determine subsequent interventions in order to improve our current practice.
Materials and Methods: We collected data for 27 children age range (10 days-16 years, mean 18 months), undergoing cardiac procedures. The following data was collected: shower/bath on day prior to surgery, completion time of intubation of line insertion and foley catheter positioning, time of incision, types and timing of antibiotic administration and type of surgical preparation.
Results:

	INTUBATION	LINE (MIN)	INCISION	TIME 1ST ANTIBIOTIC GIVEN	TIME 2ND ANTIBIOTIC GIVEN
Mean time from patient arrival in anesthetic room	13.8 min	48.9 min	74.8min	41.5min	51min

Conclusions: The data collection on antibiotic timing is part of a project to reduce SSIs. Other interventions include preoperative washing on the ward, and improved wound surveillance. Currently the mean timing of our antibiotic administration falls within the desired range of 60 minutes from incision time, however we have noticed and extreme variations among different operators.

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Insulin Resistance: Between Myth And Reality

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Background: Almost invariably, during the last two decades it was stated that type 2 diabetes appears as a consequence of increased peripheral insulin resistance (considered to be the primordial factor), associated with a beta cell defect (considered to be secondary both chronologically and as pathogenic importance). There are perhaps few diabetes cases that evolve with a normal beta cell mass. Leaving behind the actual definition/classification of diabetes, we state that the diabetic syndrome is unitary by the decrease of the beta cell mass, considered to be a *sine qua non* condition for the decompensation of blood glucose regulation. The decrease of the beta cell mass is explained wither by an increased apoptosis or by a decreased regeneration or by an association of both mechanisms. Even if the studies of islets obtained from different diabetes animal models indicated an important capacity of regeneration, however, in human diabetes during adult life this regeneration seems to be quasi-inexistent and thus incapable to compensate an increased apoptosis. The cause of increased apoptosis in diabetes could be related to the secretory beta cell dysfunction expressed by the increase of proinsulin and pro-amylin incompletely processed in the endoplasmic reticulum of these cells. Both increased proinsulin and the amiloid transformation of amylin could trigger the pro-apoptotic beta cell mechanisms. The decrease of the beta cell mass is usually slow and blood glucose decompensation appears only when >50% of the initial beta cell mass is destroyed. Increased proinsulin can interfere with beta cell regeneration while the amiloidogenic transformation of amylin can lead to increased beta cell apoptosis mediated by the endoplasmic reticulum. Genetic studies based on the classical candidate gene methods as well as the modern studies based on the Genome Wide Scan (GWS) techniques, managed to identify a dozen genes involved in T2DM pathogenesis, almost all being somehow related to beta cell function. The assiduous investigation of peripheral insulin resistance genes had a predictably failure since an abstract concept based on mathematical equations (as is insulin-resistance) cannot be localized in the real human genome. Since the increasing prevalence of diabetes mellitus has to be explained by the intervention of some environmental factors (increase of caloric intake, especially of animal lipids and decrease of physical exercise, both capable to influence the transcription of some genes), the genetic factor (rather epigenetic) involved refers to the genetically determined limits of the complex mechanisms that ensure the energetic homeostasis of the human body. The constant surplus of fuels from the human energetic system rises problems of adaptation that were not encoded in the original genome. Inclusion of insulin resistance in this disorder seems to be improper.
Conclusions: Peripheral insulin-resistance exists and can be well illustrated by the absence of insulin receptors in the rare forms of extreme insulin-resistance with a well defined genetic basis. The absence of a related mechanism in type 2 diabetes explains the rhetoric question of Flores J.C. form a recent review (Diabetologia 51:1100-1110, 2008): *"Where are the insulin resistance genes?"*

Synthesis and Evaluation of Highly Potent Antimicrobial Chromanyl-1,2,4-dithiazoles

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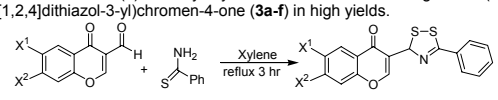
Background
A large number of hetrocycles of natural and synthetic origin exhibit valuable antimicrobial activity and include drugs such as fluconazole, ketoconazole etc. Recently, dithiazoles, have attracted considerable interest due their high fungitoxicity and of other related 1,2-dithia-heterocycles. On the other hand, a number of chromone derivatives also display antimicrobial activity. Therefore, it was decided to synthesize chromanyl-1,2,4-dithiazoles possessing both chromone and dithiazole moieties, and evaluate their anti-microbial activities.
Results:
Substituted 3-formylchromen-4-one (**1a-f**) were reacted with two equivalents of thio-benzamide (**2**) in dry xylene for 3 hours leading to 3-(5-phenyl-3H-[1,2,4]dithiazol-3-yl)chromen-4-one (**3a-f**) in high yields.



1 **2**

3 a X^1=H, X^2=H, b X^1=Cl, X^2=H
c X^1=F, X^2=H, d X^1=F, X^2=Cl
e X^1=CH_3, X^2=H, f X^1=H, X^2=Cl

These chromanyl-1,2,4-dithiazoles (**3a-f**) were evaluated *in vitro* for antifungal and antibacterial activities. Antibacterial activities (percentage growth inhibition, MIC) were determined on G+ve and G–ve bacterial starins i.e., *E.Coli*, *Pseudomonas aeruginosa*, *Shigella flexneri* and *Staphylococcus* using ciprofloxacin and chloramphenicol as positive controls. Compounds **3a,d,e,f** show very good antibacterial activity. Similarly, the antifungal activities (MICs) were determined using turbidimetry method *on Aspergillus niger*, *Geotrichum candidum*, *Candida albicans* and *Candida tropicalis* employing fluconazole as positive control. Some of the compounds display very high antifungal activity (**3f,** MIC= 5 μM & **3b,d** MIC= 28 μM); fluconazole showed MIC= 9 μM under similar conditions.
Conclusions
Compounds **3a,d,e,f** shows vary good antibacterial activity and compounds **3f,b,d** have high antifungal activity. In general, compound bearing F/Cl substitutents on chromone ring displayed high antifungal activity. These useful 'leads' can be evaluated for toxicity and developed further

New Features Of Antidepressant Drugs - Modification Of Histamine Kinetics

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Background: Antidepressant drugs (AD) exert their effects by affecting different targets. Detailed insight into the mode of action of AD could lead not only to better understanding their pharmacological effects but also to improve or expand current utilization of these drugs in clinical practice. Our studies were focused on the effects of AD, mainly amitriptyline, on histamine kinetics in experimental animals.
Methods: Different types of *in vitro* and *in vivo* studies were performed, using cat, rat and guinea pig. Animals were pre-treated with amitriptyline or other AD, given intraperitoneally. In order to investigate effects on histamine kinetics animals received histamine liberator (compound 48/80) or i.v. injection of histamine and we followed plasma and/or blood histamine concentrations. In addition, the ability of amitriptyline to interfere in histamine metabolism was studied by following effects on the two main histamine degrading enzymes, diamino oxidase (DAO) and histamine-N-methyltransferase (HNMT) measured in rat and guinea pig tissues.
Results: AD interfere with histamine system through different mechanisms. Tricyclic AD inhibit histamine release and change plasma histamine kinetics after its secretion induced by histamine liberator in the rat. Amitriptyline and other types of AD also significantly lower the increase of plasma histamine levels induced by the injection of histamine and they change the pharmacokinetic profile of the amine in feline and rat plasma and blood. Amitriptyline decreases the rate of DAO release into plasma after the heparin activation in guinea pig. It also increases DAO and HNMT mRNA expression as well as the activity of both enzymes in guinea pig tissues while in the rat it does not affect DAO activity. *In vitro* studies indicate that amitriptyline change the activity of both histamine degrading enzymes in a concentration- and animal species- dependent manner.
Conclusion: Inhibition of histamine release and increased capacity of histamine degrading enzymes in tissues renders lower concentrations of histamine in the tissues which could inhibit development of allergic/inflammatory response. Rational taking advantage of the growing knowledge on pharmacological effects of amitriptyline and other antidepressants could enrich their use in clinical practice.

Discovery Of A Novel Anti-MRSA Agent, TAK-599 (Ceftaroline Fosamil): An *N*-Phosphono Water-Soluble Prodrug For Intravenous Injection

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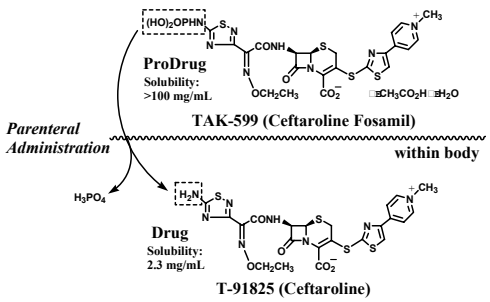
Background: Our research program was started with the aim of discovering clinically effective agents for methicillin-resistant *Staphylococcus aureus* (MRSA) infection.
Methods&Results: Considering the excellent safety and bactericidal properties of cephalosporin derivatives compared with those of the other classes of antibiotics, such as vancomycin (VCM), we examined chemical modifications of cefozopran. Considerable SAR studies led us to find potent anti-MRSA compound T-91825[1]. Although T-91825 showed insufficient water-solubility (2.3 mg/mL) for parenteral administration, our efforts were rewarded with the discovery of a crystalline form of the *N*-phosphono prodrug, TAK-599, as an acetic acid solvate[1]. TAK-599 has not only a practical level of water solubility (>100 mg/mL, pH=7), but also good chemical stability in the crystalline state and in solution. In pharmacokinetic studies, TAK-599 was converted rapidly into the active form T-91825 in blood when administered intravenously to rats and monkeys. Considering both the pharmacokinetics of TAK-599 and the potent anti-MRSA activity of T-91825, TAK-599 exhibited excellent *in vivo* anti-MRSA efficacy, superior to that of VCM.



Conclusions: TAK-599 (ceftaroline fosamil) is a promising candidate for MRSA infection. Currently, clinical trials of ceftaroline fosamil are underway, in cooperation with Forest Laboratories, Inc.

Ref 1) Tomoyasu Ishikawa, *et al*. *Bioorg. Med. Chem.* **2003**, *11*, 2427.

Dehydroepiandrosterone (DHEA) Reduced Adiposity And Insulin Resistance

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Background: We have been shown that DHEA administration decreased insulin resistance in Otsuka Long Evans Fatty (OLETF) rats, hereditary obese type 2 diabetic animals derived from Long Evans Tokushima (LETO) rats. Considering that androgen receptor (AR) deficient mice represent obesity, AR acts to prevent fat accumulation in whole body. We have further examined the effect of DHEA on telomere length and preadipocyte distribution in adipose tissue. Moreover, we assessed the role of AR in DHEA-induced growth suppression in preadipocyte.
Methods: Two mg genomic DNA isolated from adipose tissue was digested with restriction enzyme, Hinf 1/RsaI, and then Southern analysis was performed to measure telomere length. Moreover, 3T3-L1 preadipocytes were treated with DHEA and testosterone (TEST) for 24 hr, and then cell proliferation was assayed with BrdU uptake. We also assessed the effect of flutamide, AR inhibitor, and fulvestrant, estrogen receptor (ER) inhibitor, on DHEA-induced reduction of cell growth utilizing siRNA.
Results: Treatment with 0.4% DHEA for 52 wk reduced body weight and fat weight, but not food consumption, in LETO and OLETF rats. Decreased telomere length was observed in genomic DNA isolated from adipose tissue in control OLETF, which was prevented with DHEA administration. These results indicated that accelerated cell division associated with obese adipose tissue resulted in rapid telomere shorting. These results suggested that DHEA-induced growth suppression in preadipocyte may lead to attenuate subsequent differentiation, which resulted in increased preadipocyte cell number. Actually, incubation with DHEA and TEST decreased BrdU uptake to the similar extent in 3T3-L1 preadipocyte. Pretreatment with flutamide, but not fulvestrant abolished this effect. AR siRNA also inhibited DHEA-induced decreases in BrdU uptake. Moreover, we found that no difference was observed between DHEA and TEST on cell growth. Our results of inhibitor and siRNA study revealed that this effect was mediated via AR.
Conclusions: These results suggested that DHEA-induced suppression of preadipocyte proliferation, might lead to anti-obesity, anti-senescence effects and improvement of insulin resistance.

Nature's Magic: Antiteratogenic Potential Of Blue-Green Algae Spirulina

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Background: The rationale for search of a low cost, affordable antiteratogenic medicinal agent lies in the difficulties we face in the identification of human teratogen and the limitations of data derived from preclinical animal studies about teratogenic potential of a medicinal agent. Spirulina being a rich source of folic acid is a focus of interest in this respect as antiteratogenic effect of folic acid seems well established. Thus the objective of the study was to explore preventive role of Spirulina in hydrocortisone-induced cleft plate.

Methods: This study included 40 pregnant mice (10 mice per treatment, weighing 28 ± 3 g, mean ± SD). Incidence of cleft palate was compared in offsprings of mice treated as follows:
A) normal saline, B) hydrocortisone 166 mg/kg b.w., C) hydrocortisone followed by normal saline, D) hydrocortisone followed by spirulina suspension 150 mg/kg b.w. All doses were given in a volume of 1ml orally once daily through 11th to 14th day of gestation.

Results: Incidence of cleft palate in offsprings of mice treated simultaneously with hydrocortisone and spirulina was found to be significantly less compared to that in offsprings of mice treated otherwise (i.e. hydrocortisone and hydrocortisone+ normal saline). Selected parameter estimates are shown in the table (***a: p < .0001 vs. A; ***b : p<.0001 vs. B and C):

Treatment	No. of pregnant mice treated	No. of offsprings	No. (%) of offsprings with cleft palate
A: Normal saline	10	135	0 (0)
B: Hydrocortisone	10	83	69 (83.13) ***a
C: Hydrocortisone + Normal saline	10	105	84 (80) ***a
D: Hydrocortisone+ Spirulina	10	110	43 (39.09) ***b

Conclusions: Spirulina, when administered through 11th to 14th day of gestation, prevented hydrocortisone-induced cleft palate in offsprings of mice.

Title: Caffeine Sets The Brain's Excitability By Priming The Activation Of The Endogenous Cannabinoid System

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Caffeine is the world's most popular psychoactive drug and stimulant. Caffeine affects vigilance, attention, mood and arousal, and may facilitate attentiveness and some forms of learning. Caffeine's stimulant properties have been explained mainly due to its ability to interact with the adenosine receptor and induce a release of excitatory neurotransmitters, glutamate and dopamine.
Interestingly, caffeine induces dopamine and glutamate release in the shell of the nucleus accumbens, the mesolimbic brain region that is directly involved in the primary reward effects of psychoactive drugs. This suggests that caffeine may share neurochemical properties with other prototypical drugs of abuse and psychostimulants, even if caffeine does not seem to cause obvious addictive symptomes reported in all prototypical drugs of abuse such as tolerance, craving, and relapse.
Neurobiological mechanisms that cause reward feelings and pleasure involve the endogenous cannabinoid (eCB) system in the brain. Most importantly, eCB participates in the common mechanisms underlying relapse to drug re-exposure by acting on the synaptic plasticity responsible for limbic emotional memory and learning. At cellular and molecular levels, eCB binds to the same receptor as marijuana and hashish bind to, which is the type 1 cannabinoid receptor (CB1R), and disinhibits mesolimbic reward neurons by blocking their GABAergic inhibitory neurotransmission. Disinhibited reward neurons cause a pleasure and reward feeling by releasing massive doses of dopamine.
Findings from my laboratory suggest that caffeine blocks GABAergic neurotransmission by regulating the brain's endogenous cannabinoid system; more specifically, by facilitating the synthesis and release of eCB through cytosolic calcium regulatory mechanisms. This effect seems independent of previously reported caffeine's ability to interfere with GABAergic transmission by competitively binding to multiple regulatory sites of the GABAA receptor, or by disrupting chloride transporters and shifting chloride equilibrium potential to reduce its conductance.
In my presentation, I will focus on the caffeine's potential ability to interact with the brain's endogenous cannabinoid system; specifically on: 1) cellular and molecular mechanisms for the physiological interaction between these two psychoactive compounds (caffeine and eCB), and 2) how they may contribute to normal reward learning as well as maladaptive learning in drug addiction by modulating the release of GABA. This project is dedicated to new discoveries on the roles of caffeine in the synaptic function and plasticity of central neurons in health and diseases including substance abuse, maladaptive learning, and addiction; all of which are topics of great interest to a wide range of neuropharmacologists and clinical practitioners.

Hypothesis On The Physiological Significance Of The Expression Of The Drug Transporters Mdr1 And Abcg2 During Normal Tissue Regeneration And After Cancer Therapy

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Cellular multi-drug resistance (MDR), which often develops in cancer cells of patients subjected to anti-cancer treatment, remains a significant barrier to successful cancer therapy. One of the principal causes of cellular MDR development is an increased expression of ABC-transporter genes such as Abcb1 (mdr1) and Abcg2. Despite many years of intensive research, the natural biological role of mdr1 in the context of cancer has remained elusive.
While studying mechanisms of muscle regeneration we have identified the expression of mdr1 in muscle precursor cells (MPC) and its up-regulation by muscle growth factors. Further, we have shown that MPCs that express these transporters, namely, the muscle SP cells, have some characteristic in common with stem cells (Israeli et al, J. Cell Physiol 2004, 201:409-19; Benchaouir et al Exp Cell Res 2004, 294:254-68). Indeed expression of these transporters in stem cells has been described, initially in the hematopoietic system, and latter in many other tissues. Expression of drug transporters in stem cells could protect these precious cells against exhaustion. However, the mdr1ab(-/-) x Abcg2(-/-) triple-KO mouse has normal tissue viability. We therefore hypothesized that the expression of these transporters in stem cells is dispensable for normal tissue maintenance (homeostatic maintenance) but will be needed during the activation of a regeneration "genetic program" after extensive tissue damage.
To test this hypothesis in the context of muscle regeneration we created the mdr1ab(-/-) x dystrophin(-/-) triple KO mouse. This mouse that undergoes chronic muscle regeneration, has reduced muscle regeneration capacity compared to control dystrophin(-/-) mouse, and therefore in support of our hypothesis (Israeli et al, Exp Cell Res 2007, 313:2438-50).
Based on this data we proposed that the activation of mdr1 and Abcg2 expression in cells in tumor tissues that were subjected to anti-cancer treatment is part of the activation, in transformed just as in a normal tissue, of a regeneration genetic program (Israeli et al, 2005, J Theor Biol. 7;232, 41-5).
Expression in cancer stem cells of mdr1, Abcg2 and of the SP phenotype is in agreement with this hypothesis. Better understanding the role of these transporters following cancer therapy may help designing new strategies to overcome MDR in cancer.

A Series Of Antibacterial Proteins Made From Inactive Cyt-Like ORF Of *Bacillus Thuringiensis* Subsp. *Israelensis* Using The Microgene Polymerization Reaction

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Background: Insecticidal crystal proteins of *Bacillus thuringiensis* belong to two unrelated toxin families: receptors-specific Cry against insects and Cyt that lyse a broad range of cells, bacteria included, via direct binding to phospholipids. A new *cyt*-like gene (*cyt1Ca*) encoding a 60 kDa Open Reading Frame, has recently been discovered in *B. thuringiensis* subsp. *israelensis*. Neither bactericidal/larvicidal activity of *cyt1Ca* expressed in *Escherichia coli* nor hemolytic effect of His-tagged purified Cyt1Ca has been observed.
Results: In an attempt to endow inactive Cyt1Ca with Cyt1Aa-like antibacterial activity, two amino acids were replaced by QuickChange mutagenesis, E117V and N125A, so as to raise the hydrophobicity of the corresponding region, considered being the membrane-active motif. Serendipitously, the primers used for QuickChange mutagenesis displayed the intrinsic ability to expand into multiple head-to-tail tandem-repeats in the so-called Microgene Polymerization Reaction (MPR). The clones thus obtained include varying lengths of multiple repeats of the amino acid sequence VIEVLKSLLGIALA, corresponding to head-to-tail polymerization of the primer, translated in frame with Cyt1Ca. These versions of Cyt1Ca caused instant arrest in biomass growth and decreased viability upon expression in *E. coli.* Multiple insertions into the polypeptide of the non-mutated motif VIEELKSLLGINLA were also lethal. To expose toxicity of the latter motif in the original Cyt1Ca, *cyt1Ca* was appropriately truncated.
Conclusions: 1) The toxicity of the above motif is ascribed to its amphiphilic nature; toxicity was not displayed in the original Cyt1Ca because of possible motif sequestration by other parts of the protein due to its inherent folding, which may provide a safety mechanism to protect the host bacterium. 2) Combination of MPR with QuickChange can thus be exploited to design and synthesize polypeptides with antibacterial motifs, multiplied within the frame of a given protein.
Supported by an Eshkol Scholarship (to MI) and BSF Grant (to AZ).

The Role Of Lipid Rafts In Host-Pathogen Interactions: Involvement Of Lactosylceramide-Enriched Lipid Rafts In Innate Immunity And Ycobacterial Infection-

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The innate immune system is the first line of defense against invading microorganisms, including bacteria, fungi, and viruses. Phagocytes, such as neutrophils and macrophages, play important roles in the innate immune system by recognizing pathogen-associated molecular patterns (PAMPs) via pattern recognition receptors (PRRs) expressed on the cell surface, and then engulfing and eliminating pathogens. It has been suggested that membrane microdomains/lipid rafts of phagocytes are involved in these innate immune responses. Proteomic analyses of lipid rafts from plasma membranes of phagocytes have provided new insight into lipid raft-mediated processes occurring during phagocytosis. Several types of pathogens, however, have developed mechanisms to attach to and enter host cells using lipid rafts. Even after being engulfed by phagocytes, a particular group of pathogens, including Mycobacteria, Afipia felis, and Shigella, can avoid degradation by escaping from the vacuolar compartment or preventing phagosome maturation, utilizing the lipid rafts of host cells. Although the molecular mechanisms underlying these phenomena remain largely unknown, detailed understanding of these lipid raft-associated host-pathogen interactions will open new avenues for the design of effective therapeutic agents for infectious diseases.

Lactosylceramide (LacCer, CDw17), a neutral glycosphingolipid, forms glycosphingolipid-enriched microdomains, which are coupled with the Lyn protein, a member of the Src family of kinases on the plasma membrane of human neutrophils. LacCer-enriched lipid rafts act as PRRs and mediate superoxide generation, migration, and phagocytosis through the binding of ligand to LacCer. In this talk, we will discuss the membrane lipid raft-associated immune functions of phagocytes, focusing on the molecular mechanisms of LacCer-enriched lipid raft-mediated phagocytosis. We also introduce evidence indicating that LacCer-enriched lipid rafts may be involved in escape mechanisms by which Mycobacteria escape from killing by human neutrophils.

Using Chimeras With C-Terminal Tails Of Noss For Probing Of The Electron Traffic In CYPOR

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The three NOS isoforms contain unique sequences that modulate electron transfer: the calmodulin-binding sequence, the C-terminal extension, and the autoregulatory loop in the FMN-binding module of the reductase domain. In the present studies, we have transfered the control conferred by the C-termini of NOS to NADPH-cytochrome P450 oxidoreductase (CYPOR), which does not contain any of these regulatory elements. The effect of the addition of the C-terminal sequences, specific for each isoform of NOS (21-mer, 33-mer, and 42-mer for iNOS, nNOS, and eNOS, respectively), on the catalytic activity and properties of CYPOR was determined. The aim was to ascertain the possible evolutionary origin of NOS and to address the effect of new peptide recruitment on the development of new functions for CYPOR. Compared to the soluble CYPOR construct to which each of the C-termini was attached by genetic engineering, CYPOR-iNOS (+ iNOS 21-mer) was ~20% inhibited, CYPOR-nNOS (+ nNOS 33-mer) was ~26% inhibited and CYPOR-eNOS (+42-mer) was ~42% inhibited. While similar reduction in 2,6-dichlorophenolindophenol activities was obtained, ferricyanide reduction was affected much less to negligibly. In addition examination of the kinetic constants showed no significant changes in K_m for NADPH (1.88 ± 0.49 to 2.55 ± 0.48μM) at 100μM cytochrome *c* or for cytochrome *c* (19.22 ± 2.13 to 27.77 ± 2.43μM) at 50μM NADPH for all of the constructs. However, reduction of molecular O_2 was increased by the addition of C-terminal sequences, suggesting a shift in the rate-limiting step caused by interference of electron flow between FAD and FMN by the extension of the C-termini over the FAD-FMN interface in the NOS isoform structures. This conclusion has been supported by the published structures of CYPOR and of the nNOS reductase.

The modulation of CYPOR by the addition of the NOS C-termini is also supported by flavin reoxidation and fluorescence-quenching studies and antibody recognition of the C-terminal extension. These experiments support the origin of the NOS enzymes from modules consisting of a heme domain and CYPOR or ferredoxin-NADP(+) reductase- and flavodoxin-like subdomains that constitute CYPOR, followed by further recruitment of smaller modulating elements into the flavin-binding domains.

Supported by project MZO 00064165

Key Aspects for the Compilation and Enhancement of a Comprehensive Chemogenomics and Drug Discovery Compound Screening Collection

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The NIBR (Novartis Institutes for BioMedical Research) compound collection enrichment and enhancement project integrates corporate internal combinatorial compound synthesis and external compound acquisition activities in order to build up a comprehensive screening collection for a modern drug discovery organization. The main purpose of the screening collection is to supply the Novartis drug discovery pipeline with hit-to-lead compounds for today?s and the future's portfolio of drug discovery programs, and to provide tool compounds for the chemogenomics investigation of novel biological pathways and circuits. As such, it integrates designed focused and diversity-based compound sets from the synthetic and natural paradigms able to cope with druggable and currently deemed undruggable targets and molecular interaction modes. Herein, we will summarize together with new trends published in the literature, scientific challenges faced and key approaches taken at NIBR to match the chemical and biological spaces.

The Outer Membrane Vesicles Of An Antarctic Bacterium *Pseudomonas Syringae* Lz4W

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Background: Outer membrane vesicles are constantly discharged from Gram –ve bacteria during cell growth. The outer membrane vesicles perform several biological functions, such as DNA transfer, and protein delivery to eukaryotic or prokaryotic cells. Characterization of these vesicles may facilitate the design of protein, DNA or other drug carriers. Studies on cold adapted bacteria might further facilitate the detection of biotechnologically important molecules such as enzymes. The main objective of these
studies is to characterize the naturally releasing vesicle from bacteria, that may help to design new vesicle with required protein /drug molecules and use them as transporters. Membrane vesicles of the Antarctic bacterium *Pseudomonas syringae* Lz4W were prepared and characterized.
Methods: The bacterium was grown at 22^0 C harvested the cells by centrifugation, the supernatant was filtered through a 45 μm filter, and the filtrate was ultra centrifuged, to obtain the vesicles as a pellet. The proteins of these vesicles were identified with the help of mass spectrometry and using NCBI data base of the *Pseudomonas* sp.
Results: Transmission electron microscopy revealed that the size of the proteo-lipids ranged from 90 to 160nm.The vesicles contained ~10 kb DNA fragment. Vesicle proteins were fractionated on a 10% SDS-gel and the trypsin digests of the bands were analyzed by liquid chromatography-matrix assisted laser desorption/ionization. The vesicles contained about 100 proteins. The subcellular proteins present were from cytoplasm, inner membrane and outer membrane.
Conclusions: 1) The outer membrane vesicles of the *Pseudomonas syringae* Lz4W were prepared and characterized. 2) The proteins identified from these vesicles were from different locations of the bacterium and have diverse functions such as transport, metabolism, antimicrobial, anti parasitic and several other functions.

Apatone®, A Combination Of Vitamins, With *In Vitro*, *In Vivo* And Clinical Effectiveness Against Prostate Cancer

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Background: When vitamin C (VC) and vitamin K_3 (VK_3) were combined in a 100:1 ratio (Apatone®), the cytotoxicity against DU145 and PC-3 prostate cancer cells was potentiated 4- to 20-fold. Oral Apatone also significantly reduced the growth rate of the solid tumors in nude mice ($p < 0.05$) without inducing significant bone marrow toxicity, changes in organ weight or pathological changes in these organs. Electron micrographs revealed tumor cell death by autoschizis (self-excision of organelle-free cytoplasmic pieces which left an intact, pyknotic, nucleus surrounded by a narrow rim of cytoplasm which contained most organelles). Apatone treatment induced a G_1/S and G_2/M block, diminished DNA synthesis, increased hydrogen peroxide production, decreased cellular thiol levels and increased intracellular Ca^{2+} levels. Electrophoretic analysis of DNA revealed a spread pattern of degradation due to the sequential activation of DNase I and DNase II and was independent of cytochrome C release and caspase-3 activation. The current study was designed to evaluate the safety and efficacy of oral Apatone administration in the treatment of prostate cancer in patients who failed standard therapy.
Materials and Methods: Seventeen patients with 2 successive rises in PSA after failure of standard local therapy were treated with (5,000 mg of VC and 50 mg of VK_3 each day) for a period of 12 weeks. Prostate Specific Antigen (PSA) levels, PSA velocity (PSAV) and PSA doubling times (PSADT) were calculated before and during treatment at 6 week intervals**.** Following the initial 12 week trial, 15 of 17 patients opted to continue treatment for an additional period ranging from 6 to 24 months. PSA values were followed for these patients.
Results: At the conclusion of the 12 week treatment period, PSAV decreased and PSADT increased in 13 of 17 patients ($p \leq 0.05$). There were no dose-limiting adverse effects. Of the 15 patients who continued on Apatone after 12 weeks, only 1 death occurred after 14 months of treatment.
Conclusion: Apatone showed promise in delaying biochemical progression in this group of end stage prostate cancer patients.

Alpha1-Antitrypsin Augmentation Therapy: New Insights Into The Molecular Basis Of Efficacy

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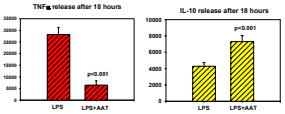
Background: The augmentation therapy with human purified plasma α1-antitrypsin (AAT) is approved for emphysema patients with severe inherited AAT deficiency. The major concept behind augmentation therapy is that a rise in the levels of blood and tissue AAT will restore protease/anti-protease balance and will protect lungs from the destruction by neutrophil elastase. However, AAT also appears to function as an endogenous inhibitor of inflammatory cytokines, apoptosis and anthrax toxin induced cytolytic activity. In an in vivo model of murine islet cell allograft rejection, AAT blocked rejection when used as a single agent. Our aim is to characterise the pleitropic functions of AAT.
Methods: Human blood monocytes were isolated from blood donors (n=65). Cells were stimulated with lipopolysaccharide (LPS, 10 ng/ml, J5 Rc mutant), 10–50μM rolipram or forskalin, or 0.5 mg/ml AAT, separately and in combination for 30 min and 1 and 18 h at 37°C, 5% CO_2. Cells and cell culture supernatants were analyzed to determine tumor necrosis factor-α (TNFα) and IL-10 expression and release by using enzyme-linked immunosorbent assays and Quantitative Real Time Reverse Transcription. Total cAMP levels and protein kinase A (PKA) activity were determined using commercial kits.
Results:



1) AAT inhibited LPS-stimulated TNFα release and expression, and enhanced IL-10 release (see Figure);
2) In monocytes pre-treated with LPS for 1 h followed by addition of AAT (0.1–4 mg/ml) for 2 min, cAMP levels increased 82–190%, $p<0.001$;
3) Pre-treatment of the monocytes with rolipram alone resulted in an 87% ($p < 0.01$) increase in cAMP level and caused an augmentation in cAMP levels in response to AAT (52% increase, $p < 0.01$, $n = 3$ experiments) compared with rolipram alone;
4) Both AAT (0.5 mg/ml) and forskolin (as positive control) increased PKA activity 213 and 256% ($p < 0.001$), respectively, compared with control.
Conclusions: 1) The anti-inflammatory activities of AAT *in vitro*, namely inhibition of endotoxin-stimulated TNFα and enhancement of interleukin-10 in human monocytes, are mediated by an elevation of cAMP and activation of cAMP-dependent PKA; 2) Elucidation of the pleitropic functions of AAT may help to improve efficacy of augmentation therapy and to design novel therapeutic drug candidates to exploit the therapeutic potential of AAT-like molecules.

Clusters of Free Radicals from Dihydroartemisinin Cure Several Parasitic and Viral Diseases. They Attack Cancers by Activating a Series of Apoptotic Pathways and by Causing Strong Angiogenesis Inhibition

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Dihydroartemisinin (DHA); a reduced form of artemisinin; is a sesquiterpen which posses peroxide in the form of seven-membered ring and a lactol which makes it a highly energetic and unstable molecule but at the same time this instability makes it useful for number of diseases including actions against parasites such as malaria and schistosomiasis, also it is effective agains bacteria, fungi and some selected virus. DHA is unstable so that it is hardly suitable for use pharmaceutically as its own, certain derivatives are made to increase the stability and ease in formulation. Artesunate, an ester of DHA and artemether, a methyl ether of DHA are common in use and further research is going on to explore other derivatives.

The mechanism of artemisinin is complex. It is reported that peroxide moiety is responsible for its reactivity. Upon contacting with iron of the cell it generates free radicals which are believed to react with the biomolecules of parasites. Based upon this mechanism the synthetic peroxides have also been investigated but none of them has been in market upto now. Variety of other possible mechanisms are also proposed that included targteting the SERCA or its effect on immune system.

Artemisinin derivatives can also selectively kill cancer cells and retard the growth of tumour. These derivatives induce the apoptesis via generation of reactive oxygen species (ROS). This mechanism is different from common anticancer drugs such as Doxorubicin and therefore Artemisinins could be used in paralled to doxorubicin or other DNA intercalators or Doxorubicin-resistant cells. The induction of apoptosis by artemisinins was shown for human KS-IMM Kaposi sarcoma cells, whereas normal endothelial cells don't undergo apoptosis in the presence of artemisinins.

Artemisinin derivatives are also reported to inhibit the antiogenesis which is required by tumor cells to get the oxygen and nutritions. Artemisinin derivatives significantly inhibit angiogenesis in a dose dese-dependent manner. Recently clinically relavent anti-tumour effects were clearly demonstrated in man.

Is Levodopa The Magic Bullet For Parkinson's Disease?

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Background: Much progress has been made since James Parkinson first described the disease in 1817, but Parkinson's disease (PD) continues to be one of the most common causes of disability, particularly among the elderly. Our understanding of the etiopathogenesis of PD has improved markedly with the discovery of genetic causes and with growing knowledge of mechanisms underlying neurodegeneration in the substantia nigra and resulting depletion of dopamine and other neurotransmitters (Pan et al. Brain 2008;131:1969-78). Dramatic improvement in the cardinal signs of PD, tremor, rigidity, akinesia, and postural instability (TRAP) and other motor symptoms, in response to levodopa has been recognized since 1961, when Birkmayer and Hornykiewicz (Wien Klin Wochenschr 1961;73:787-8) first treated parkinsonian patients with the dopamine precursor.
Methods: Evidence-based literature on the efficacy and safety of levodopa was critically reviewed and analyzed in the context of long-term experience. Videos of patients treated with levodopa will be presented to illustrate its efficacy and motor complications.
Results: The therapeutic options for patients with PD have been expanding with the introduction of dopamine agonists, MAO-B inhibitors, and other medical and surgical strategies, but levodopa continues to be the "gold standard" and is considered to be the most effective drug in the symptomatic treatment of PD. Psychiatric and motor complications, including fluctuations and dyskinesias, can be managed effectively until the advanced stages of the disease. There is no evidence of levodopa-related neurotoxicity from in vivo studies or long-term clinical experience.
Conclusions: Levodopa is not only the most effective drug in the treatment of PD, but its introduction revolutionized treatment of neurodegenerative disorders and transformed Neurology from a primarily a diagnostic specialty to a therapeutic discipline and as such it may be considered "the magic bullet" in Neurology. Despite its extraordinary impact on the quality of life of patients with PD, there are, however, many limitations to levodopa, including the various acute and chronic complications and its lack of efficacy in certain "axial" motor signs, particularly freezing of gait and postural instability, and in most non-motor symptoms associated with PD, such as behavioral, cognitive, sensory, autonomic and sleep disorders (Jankovic J. J Neurol Neurosurg Psychiatry 2008;79:368-76). Because of the broad diversity of symptoms associated with PD and the growing recognition that non-dopaminergic neurotransmitters are also involved in PD, future therapies will likely involve not just a single bullet but a shot-gun approach targeting many systems.

Sulfasalazine Revisited: A Multi-Targeted Magic Bullet

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Background: Sulfasalazine (SSZ) is commonly prescribed for the treatment of patients with chronic inflammatory diseases, such as those with rheumatoid arthritis (RA). Here we report on the novel mechanisms of action of SSZ, SSZ-drug interactions with the folate antagonist methotrexate (MTX), and mechanisms of acquired resistance to SSZ.
Methods: In vitro cell line model systems of immune effector cells (T-cells, macrophage cells) implicated in the pathophysiology of RA were used to evaluate anti-inflammatory properties of SSZ after short-term drug administration or after chronic exposure to stepwise increasing concentrations of SSZ, which provoked acquired resistance to SSZ.
Results: SSZ proved to be a potent inhibitor (IC50: 0.55 mM) of the production of pro-inflammatory cytokine tumor necrosis factor α via inhibition of the nuclear transcription factor NFκB. This capacity was 3-fold reduced for cells with acquired resistance to SSZ. The mechanistic basis underlying SSZ resistance involved the overexpression of a cell membrane-associated drug efflux transporter, ABCG2, which reduces intracellular SSZ concentrations. Further studies revealed that SSZ was a potent, non-competitive inhibitor (IC50: 0.3 mM) of the Reduced Folate Carrier (RFC), the dominant transporter for the cellular uptake of MTX and natural folates. Concurrently, this SSZ-RFC interaction provokes an intracellular folate depletion that could further enhance the therapeutic effect of MTX in SSZ+MTX drug combinations, but only when SSZ administration precedes and is spaced in time from MTX administration. Finally, chronic exposure of cells to stepwise increasing concentrations of SSZ markedly increased the cellular sensitivity for the glucocorticoid (GC) drugs prednisolone and dexamethasone. In fact, after SSZ exposure, primary GC-sensitive T cells displayed 10-20 fold greater sensitivity to GCs, while primary GC-resistant macrophage cells resumed full GC-sensitivity due to a greatly enhanced upregulation and stabilization of the GC-receptor-α, facilitating enhanced GC-induced apoptosis.
Conclusions: SSZ not only elicits potential anti-inflammatory activity as a direct inhibitor of the NFκB pro-inflammatory signalling pathway, by targeting other cellular processes (a.o. folate and glucocorticoid metabolism), SSZ allows a rational utilization in drug combinations.

Comparison of the Pharmacodynamics of Imipenem in Patients with Ventilator-Associated Pneumonia following Administration by 2 h or 0.5 h Infusion

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Background: The time that concentrations in plasma are above the MIC (*t*>MIC) is the pharmacokinetic/pharmacodynamic parameter correlating with the therapeutic efficacy of β-lactam antibiotics. The aim of this study was to compare the *t*>MICs of imipenem between administration by a 2 h infusion with a 0.5 h infusion.
Methods: The study was a randomized three-way crossover in nine patients with ventilator-associated pneumonia. Each subject received imipenem in three regimens consecutively: (i) 0.5 h infusion of 0.5 g every 6 h for 24 h; (ii) 2 h infusion of 0.5 g every 6 h for 24 h; and (iii) 2 h infusion of 1 g every 6 h for 24 h.
Results: Following the 0.5 h infusion of 0.5 g of imipenem, the percentages of the *t*>4MICs of 4, 2, and 1 mg/L were 20.32% ± 9.32%, 44.11% ± 16.40%, and 64.67% ± 20.56% of a 6 h interval, respectively. For the 2 h infusion of 0.5 g of imipenem, the percentages of the *t*>4MICs of 4, 2, and 1 mg/L were 17.71% ± 19.27%, 53.75% ± 19.30% and 76.54% ± 17.36% of a 6 h interval, respectively. For the 2 h infusion of 1 g of imipenem, the percentages of the *t*>4MICs of 4, 2, and 1 mg/L were 60.26% ± 23.96%, 77.78% ± 20.11% and 93.35% ± 8.26% of a 6 h interval, respectively.
Conclusions: 1) The 2 h infusions of imipenem resulted in greater *t*>MICs than the 0.5 h infusion. 2) For infections caused by pathogens with high MIC, a 2 h infusion of 1 g of imipenem every 6 h can provide plasma concentrations above the MIC of 4 mg/L for 60% of a 6 h interval.

Decontamination of Cardiovascular Allografts in European Homograft Bank (EHB). Comparison of different Antibiotic Cocktails in low Concentration low Temperature Conditions

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Background: Heart valve and vascular allografts have been processed in the EHB since 1989 and 1991, respectively. Low temperature (+4°C), low concentration cocktail of four different antibiotics (cefoxitin, lincocin, vancocin and polymixin B) during respective 24 and 48 hours for heart-beating and non heart-beating donors was classical decontamination protocol. We have compared the efficiency of different incubation periods as well as antibiotic cocktails (4 versus 3 antibiotics) in allograft decontamination.
Methods: Donors were of three categories: living, heart-beating multiorgan donors **(MOD)** and recipients of heart transplantation **(RHT)** and non heart-beating **(NHBD)** or cadavers with age up to 65 years. Tissue preparation is performed in grade A laminar flow. First part of the study included 948 allografts from 541 donors, assessing decontamination efficiency depending on duration of incubation (below 30 versus above 30h). Second part inluded 80 donors during first step and 366 other donors during second step for allograft incubation in classical (4 antibiotics) versus modified cocktail (3 antibiotics). Bacteriological examination is carried out during dissection (a-sample), following the period of incubation (b-sample) and just before cryopreservation (c-sample). Examination for aerobe and anaerobe bacteria is carried out during 14 days at 37°C in two enriched culture media, according to the European Pharmacopoeia. For fungi and yeasts the medium was enriched with trypto-caseine and soja at 20-25°C during 14 days. Different data groups are statistically compared using Fisher's exact test considering p value of <0.05 as significant.
Results: Initial contamination rate during first part of the study was 36.4% (NHBD-78.1, MOD-36%, RHT-21.6%) with significantly higher incidence for NHBD than HBD group (p<0.001). Final sterility rate was 94.0% (MOD-95.4%, RHT-96.8%, NHBD-86.3%, p>0.5). Difference between contamination rate in the beginning and end stage of allograft processing was significant (p<0.001). Difference between duration of incubation and decontamination rate was not significant (p<0.1). During second part of the study, among 80 donor tissues of first step, 23.75% were initially contaminated (RHT-0.0%, MOD-21.66%, NHBD-33.3%). Tissues incubated in classical cocktail of antibiotics were sterile in 93.75% and those in modified cocktail 100% (p=0.058). During second step, initial contamination rate of group one was 25.54% and group two 30.77%. Final decontamination rate was 90.22% and 90.11% for group one and two, respectively (p=0.8).
Conclusions: Initial tissue contamination was significantly higher among NHBD than HBD. Effectiveness of decontamination rate in the end of processing was significantly higher in all donor groups. However, highest final contamination rate was among NHBD group. Duration of incubation did not influence final sterility rate of allografts. Modified antibiotic cocktail was as efficient as classical one in allograft decontamination.

A New Physiological Role for Dopamine and its Transporter in the Pituitary: Induction of Prolactin Cells Apoptosis at Weaning.

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Background: In the rat pituitary gland, cessation of lactation causes a massive loss of prolactin (PRL) cells, eliminating the surplus of cells coming from previous proliferation occurring during pregnancy and lactation. The factors and mechanisms involved in this phenomenon were unknown, but our study provides the first evidences that dopamine (DA) plays a key role in this process.
Results: We tested the pro-apoptotic effect of DA on pituitary primary cells from virgin, lactating, and post-lactating rats. By measuring several apoptotic markers as mitochondrial membrane potential loss, caspase-3 activation, and nuclear fragmentation, we show that DA induces apoptosis specifically in PRL cells from post-lactating rats.
Surprisingly, although the DA receptor (D2R) expressed in PRL cells has been linked to inhibition of cell proliferation, and D2R agonists are used in therapy against pituitary tumors, the D2R is not involved in DA induced-apoptosis in our context. We then determined that this effect was mediated by the DA transporter (DAT), as revealed by a pharmacological study corroborated by detection of the DAT expression exclusively in PRL cells from post-lactating rats.
In the same time, we also observed the expression of tyrosine hydroxylase (TH, the major enzyme of the DA synthesis) in post-lactating PRL cells which was accompanied by an increase in DA content in the AP gland of post-lactating as compared to virgin rats. Finally, we observed that cells expressing TH co-expressed DAT and cleaved caspase-3.
Finally, we studied in a PRL cell line model (GH3 cells) the DA-induced apoptotic pathway and showed that, as described in the neuronal model, transported DA induces oxidative stress, leading to stimulation of pro-apoptotic proteins involved in the mitochondrial pathway (Bax, Cytochrome c) and finally to caspase activation.
Conclusions: These findings show that DA may play an important role in lactotroph regression during the post-lactation period by inducing apoptosis. The fact that this process requires DAT and TH expression by lactotrophs themselves suggests that it may be "autocrine" in nature. This mechanism, already described in neuronal model of parkinson desease, could be the first physiological example of a regulatory expression of DA and DAT to specifically induce apoptosis. A better understanding of the mechanisms inducing these regulations could help us to explain pituitary tumors formation (particularly prolactinoma), no convincing explanation having never been caracterised so far.

Combined Targeting of IL6 and VEGF Potently Inhibits Glioma Growth and Invasiveness

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Background: Interleukin-6 (IL6) and Vascular Endothelial Growth Factor (VEGF) are abundantly produced by glioma cells and contribute to malignancy by promoting angiogenesis-dependent proliferation and resistance to apoptosis. We compared the effect of RNA interference against IL6 and VEGF on the growth of experimental glioma.
Methods: U87 human glioma cells with IL6, VEGF or IL6/VEGF knockdown were analyzed in vitro and implanted on the chick chorio-allantoic membrane (CAM). Tumor growth was monitored by biomicroscopy and immunohistology. Molecular response of tumor cells to single or combined treatment was studied by transcriptomic profiling.
Results: *In vitro*, IL6 knockdown had no effect on proliferation but substantially enhanced invasion. *In vivo*, IL6 knockdown reduced growth and vascularization of the tumors but revealed tissue-invasion properties of the resistant tumor cells. By contrast, IL6/VEGF knockdown not only had a greater inhibition effect on the tumor mass and on angiogenesis but also prevented resistant cells to invade the host tissue. Interestingly, cell cycle promoting genes as well as modulators of blood vessel morphogenesis were specifically downregulated in the double knockdown, illustrating a synergistic effect of the combined treatment. Invasive behavior of tumor cells under IL6 inhibition was explicited at the molecular level with the induction of known marker genes (e.g. CYR61, IL6ST).
Conclusions: Our results show that treatment of glioma with a combination of IL6 and VEGF inhibitors brings synergistic antitumoral benefit and reduces the risk of activating major pathways of cell survival, proliferation and invasiveness in remaining tumor cells that may be promoted by using IL6 inhibitors alone.

Impact of Cisplatin potentiation by Cytarabine in the 5-FU-CDDP regimen for dismal- prognosis head and neck cancer (HNC) patients; a meta-analysis of 3 local trials involving 492 patients.

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Further to our randomized study demonstrating response and survival benefit for Cytarabine (CAR) 1000 mg/m2 potentiating Cisplatin (CDDP) in the standard 5-FU-CDDP regimen (Eur J Cancer 2002) in dismal-prognosis HNC patients (unresectable T4 N2c-3 or relapsing or metastatic) two further studies were done. One compared potentiation with CAR 500 mg/m2 versus 1000 mg/m2; the other compared the CAR 500 mg/m2 with 5-FU administered as bolus versus continuous infusion (RR and OS were identical in both studies). The present report is a meta-analysis of the 3 trials with response and survival as main issues. The three studies included a total of 482 patients. Cohort 1 received the standard 5-FU-CDDP regimen (83 pts), Cohort 2 CAR-1000-5-FU-CDDP (153 pts) and Cohort 3 CAR-500-5-FU-CDDP (246 patients). All there regimens were applied both in palliative and neoadjutvant setting, the neoadjuvant preceding radiotherapy with 70 Gy. RR and PD rates were assessed on evaluable patient basis and survival on intend-to treat basis. Statistical analysis included the chi-square test, the log-rank test, determination of the death hazard radio and Cox regression analysis. Significance was assessed by the t-test with Bonferroni correction. The RRs were significantly higher in CAR-potentiated Cohorts (Cohort 1 44%, Cohort 2 62%, Cohort 3 66%, p=0,0031) and PD rates in the standard 5-FU-CDDP Cohort (Cohort 1 43%, Cohort 2 21%, Cohort 3 15%, p<0.001. The median survival in Cohort 1 was 7 months and, in Cohorts 2 and 3, 11 months. The one and two years survivals were for the Cohort 1 26% and 6%, for Cohort 2 42% and 14% and for the Cohort 3 44% and 24%. The difference in survival with the log rank test was highly in the favor of both CAR-potentiated Cohorts (p<0.0001) with the power of over 90% for p=0.01. Cox regression analysis showed that both performance status, primary tumor localization and treatment schedule were significant predictors of survival. The highest impact on survival had the administration of the CAR-potentiated regimens with a death hazard ratios of 0.58 an 0.53 (CI respectively 0.44-0.77 and 0.40-0.70) as compared to standard 5-FU-CDDP regimen. Potentiation of CDDP by CAR improves both RR and survival in dismal-prognosis HNC patients. The choice of the neoadjuvant regimen prior irradiation is crucial in judging its benefit impact in otherwise dismal-prognosis HNC patients.

Therapeutics: A New Control Strategy Using The Doses As Well As The Serum Concentrations To Optimize Learning About The Patient While Treating Him/Her At The Same Time

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Our laboratory has developed a new dual controller that combines, for the first time, particle filtering for nonlinear estimation with the Iteration-in-Policy Space (IPS) algorithm for approximating the Stochastic Dynamic Programming (SDP) equations of Bellman. Dual controllers optimally proportion their effort between controlling the patient, and actively probing the patient to extract useful information. This probing action is known to systematically improve controller performance compared to controllers that do not probe. Translated into clinical practice, dual controllers provide better drug regimen design and less variability in patient response.

We tested the method first by applying it to a problem of controlling a model of a swinging pendulum. That model provides a non-trivial yet physically interpretable example. The model contains process noise (a child's swing blows in the wind) and is controlled by pulling and pushing on it at allowable times (analogous to the doses). The controller goal is defined by having the pendulum achieve a prescribed excursion at a specified time. The problem is made difficult by taking both the length and mass of the pendulum to be unknown parameters, the sign of the control influence parameter (push or pull) to be unknown, and the pendulum position measurements to be in error (i.e., measurement noise). These complexities make the problem challenging, yet similar to the clinical problem of actively controlling a 1 or 2 compartmental aminoglycoside model.

Results of a Monte Carlo study of the pendulum are shown in Table 1. The Heuristic Certainty Equivalence (HCE) controller, using Maximum Aposteriori Probability (MAP) Bayesian adaptive control, incurs an expected cost of 18.8 compared to 16.5 for the active 1-IPS(HCE) algorithm. This represents an improvement of 2.3 expected cost units. On the same example, the OLF policy using multiple model (MM) Bayesian control incurs a cost of 15.9 compared to the 14.9 for the 1 IPS(OLF) algorithm.

CONTROLLER PERFORMANCE COMPARISON		
Control Law	Expected Cost	CPU Time (s)
HCE	18.794	.05
1-IPS(HCE)	16.536	20
OLF	15.874	.05
1-IPS(OLF)	14.873	20

Table 1: Simulation results comparing stochastic controller costs incurred on the pendulum control example

The particle filter - IPS algorithm improves upon standard HCE and OLF stochastic control policies. These results are encouraging, and have specific importance in the context of pharmacokinetic applications, as most current drug dosage designs are either of the HCE (cf., [1]) or OLF (cf., [2,3]) type. A manuscript [4] has been submitted.

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Therapeutics: New Protocols For Instituting Continuous Intravenous Vancomycin Therapy

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Wysocki et al [1] showed that continuous intravenous (CIV) vancomycin therapy did as well as intermittent (IIV) therapy, with similar clinical outcomes and safety. Target serum concentrations were reached in 36 ± 31 hrs for CIV versus 51± 39 hrs for IIV. Ten day treatment cost was $321± 81 for CIV versus $454 ± 137 for IIV. They concluded that CIV may be a cost-effective alternative to IIV.

We were impressed with their approach and began to create treatment protocols for CIV, and to compare them with IIV every 12 yours over 1 hour. For a representative 65 year old man, 70 in tall, 70 kg weight, serum creatinine of 1.0 mg/dL. For a target trough goal of 15 ug/ml, the ideal IIV regimen was a 1929 mg first dose, a 978 mg second dose, tapering down to 831 mg q 12 h for the last four doses. Total 10 day AUC was 5329 ug*hr/ml. Peak concs were 83 ug/ml for the first dose, 56 for the second, tapering down to 50 ug/ml after 60 hours. Trough concs were 15 ug/ml throughout, as shown below. The total daily dose approached 1662 mg/day.

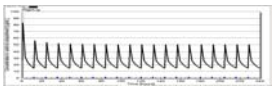

Figure 1. Plot of predicted serum concentrations on IIV therapy q 12 h for 10 days.

In contrast, using the MM-USCPACK clinical software [2,3], for the same 15 ug/ml continuous concentration, the ideal CIV regimen consisted of a loading dose of 540 mg over 3 hours, then 271 mg for the next 3 hours, then 948 mg over the next 18 hours to finish out the first day. Subsequent doses were 1120 mg for day two, tapering down to 1056 mg for the last day. Total 10 day AUC was only 3133 ug*hr/ml, only 59% of the IIV AUC. All serum concs were 15 ug/ml, as shown below. For such a typical patient, this regimen can be approximated as 500 mg for the first 3 hrs, 250 mg for the neat 3 hours, 1000 mg for the next 18 hours. And then 1000 mg daily thereafter. Predicted concentrations at the end of the first 500 mg infusion are 13.9 ug/ml, after the 250 mg infusion are 13.8 ug/ml, after the 18 hour 1000 mg infusion are 15.4 ug/ml, and thereafter are about 14 ug.ml. This is therefore a very practical approximation of the ideal infusion regimen. A plot of these predictions is shown below. Effective concentrations are now achieved after only three hours instead of the longer times found by Wysocki et al [1].

As vancomycin is not a concentration dependent drug, usual target trough goals are about 5-6 times the anticipated MIC of the infecting organism. Above this, bacterial kill plateaus off, and higher peak concentrations are not needed. As shown by Wysocki et al., CIV vancomycin is just as effective, saves drug, and visibly reduces the risk of toxicity. The author has seen two patients with aplastic anemia felt by hematology to be due to vancomycin toxicity. They had received it IIV q 12 h. It is very likely that is they had received it CIV instead, their toxicity would have been visibly less.

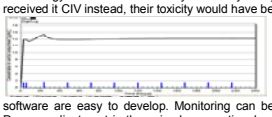

Figure 2. Plot of predicted serum concentrations on CIV therapy for 10 days.

CIV vancomycin protocols such as this one, individualized for each patient using the MM-USCPACK software are easy to develop. Monitoring can be done with a sample at 6 hours, then q AM as needed. Dosage adjustment is then simple proportional adjustment to the deviation of the serum concentration from the desired target goal.

ACKNOWLEDGEMENTS: Supported by NIH grant GM005803.

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Therapeutics: Two New Bayesian Methods For Parameter Updating In Individual Patients

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The MM-USCPACK clinical software for dosage individualization uses nonparametric (NP) pharmacokinetic models. It develops maximally precise dosage regimens to hit targets with minimum weighted squared error. It then computes the Bayesian posterior probability of each support point in the NP model. Those points fitting the patient's data well become more likely, and vice versa. However, the patient's individual model must be reasonably well represented by support points in the model. If this is not the case, poor parameter updates may be obtained.

1. We have now developed a new hybrid (H) Bayesian approach to such updating. It begins with conventional maximum aposteriori probability (MAP) Bayesian estimation. However, extra support points are then added in the region of the MAP estimator to augment the population model for the data it will now receive. This provides a much richer set of support points in that area. It also provides a richer and safer approach to individualized NP Bayesian parameter estimation and maximally precise dosage design. This new approach is now being implemented in our clinical software.

2. We have also developed a new sequential interacting multiple model (IMM) Bayesian approach to best track and estimate patient parameter distributions as they change in unstable patients who have changing parameter values with their changing clinical status. All current Bayesian updating procedures assume that there is only one set of fixed parameter values that best fit the data. The IMM Bayesian procedure is widely used in aerospace. It permits parameter values to change during the fitting procedure [1].

Results: IMM tracks drug behavior in a simulated changing patient with less than half the error of the MAP or MM procedures. It has been incorporated into the MM-USCPACK clinical software. It now has tracked the behavior of gentamicin and vancomycin significantly better than MAP and MM methods in over 130 unstable post-cardiac surgical patients. Both tools show great promise in optimizing patient care [2].

ACKNOWLEDGEMENTS: Supported by NIH grants GM068968 and EB005803,

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P2X Purinergic Receptor Modulation Of Excitatory Nociceptive Transmission Involve NMDA Receptors

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Background: The majority of trigeminal small-diameter nociceptive primary afferent fibres innervating craniofacial tissues terminate in trigeminal subnucleus caudalis (also termed the medullary dorsal horn, MDH). We have previously shown *in vivo* that central sensitisation of nociceptive neurons in deep MDH laminae can be induced by purinergic (P2X) receptor agonists and blocked by P2X antagonists applied in this area (Chiang *et al.*, 2005). Our *in vitro* studies indicate that P2X agonists act presynaptically to facilitate MDH excitatory neurotransmission (Jennings *et al.*, 2006). This study aimed to test if these P2X receptor processes involve NMDA receptor mechanisms and PKC. These are important as it addresses some of the underlying mechanisms of prolonged neuronal excitation in pain pathways.
Methods: Sprague-Dawley rat pups (9-16 days) were anaesthetised with halothane, decapitated and horizontal slices (250μm) were cut from the caudal brainstem. Whole-cell patch-clamp recordings (voltage clamped at –70mV) were made from MDH neurons.
Results: An increase in excitatory neurotransmission in deep MDH laminae, as reflected in an increase in mEPSC rate, was induced by the ATP analogue α,β-methylene-ATP (α,β-meATP, 30 μM, n=14; P<0.01) but was blocked (no change in mEPSC rate or amplitude; n=7; P>0.1) following superfusion of the NMDA antagonist AP5 (40μM). In other brain areas, phosphorylation of NMDA receptors through PKC has been shown to potentiate glutamatergic neurotransmission, so we tested if α,β-meATP activated an intracellular kinase cascade that altered NMDA receptor function in MDH α,β-meATP (30μM) applied in the presence of the kinase inhibitor staurosporine (2μM) did not affect mEPSC rate (n=7; P>0.9). Furthermore, application of the phorbol ester (PDBu; 500 nM), which potentiates PKC-induced responses, caused a 294 ± 71 % (n = 6; P < 0.05) increase in mEPSC rate without change in amplitude. However, in the presence of AP5, PDBu caused a smaller increase (50 ± 5 %) in mEPSC rate, suggesting that phosphorylation of NMDA receptors is important.
Conclusion: These results suggest that P2X receptor-mediated central sensitisation in the deep MDH is mediated via NMDA receptors. Furthermore, our preliminary data suggests Protein kinase phosphorylation of NMDA receptors is involved.

Perspective of chromatin structure on Topoisomerase II: Exclusive dynamics between the nucleosome and the drug target in *Saccharomyces cerevisiae*

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Background: Topoisomerase II (Top2) is a ubiquitous and highly conserved enzyme that serves as a target for anticancer and antibiotic therapy. Top2 introduces DNA double-strand breaks and rejoins the DNA after strand exchange to resolve topological problems. Although Top2 activity in chromosome segregation is essential for cell viability, the mechanisms of replication-independent cell killing remain to be elusive. The DNA-binding of Top2 and the DNA-Top2 covalent complex caused by Top2 inhibitors are the critical steps for its inhibition. Despite the importance of Top2 interaction with naked DNA, very little is known about its relationship with histone proteins which limit access to DNA by forming a nucleosome, a basic unit of chromatin. This study aims to understand the effect of changes in chromatin structure on Top2 activities.
Methods: We determined nucleosome density and the genome-wide distribution of Top2 and RNA polymerase II (Pol II) using high-density oligonucleotide tiling arrays. This study included budding yeast mutants defective in Top2 activity, nucleosome formation and Pol II transcription to determine their relationship. The analysis of DNA sequences was performed with genomic location data of Top2 to compare DNA motifs for Top2 binding with nucleosome positioning sequences.
Results: Top2 is localized in nucleosome-free regions in the *S. cerevisiae* genome. Mutually exclusive distribution between nucleosome and Top2 is a genome-wide phenomenon determined primarily by relocalization of nucleosome. Actively transcribed genes contain more Top2 than poorly expressed genes. However it is not simply a consequence of Pol II activity leading to an accumulation of excessive DNA supercoils. Nucleosome loss together with increased DNA binding of Top2 is a prerequisite for Pol II recruitment. This exclusive relationship in DNA binding between histones and Top2 in part depends on the anti-phase properties between nucleosome positioning DNA sequences and DNA motifs for Top2 binding.
Conclusions: 1) The binding of Top2 to DNA strictly depends on nucleosome remodeling. 2) The DNA-Top2 interaction, a key step in pharmacological inhibition of Top2, prominently occurs in the promoters of actively transcribed genes. 3) Effectiveness of Top2 inhibitors needs to be considered in the context of DNA-histone interaction.

Effect of Fish Oil with Garlic Oil Supplementation on the Anthropometric Measurements,Serum lipid Profile and Blood Pressure in Women with Hypercholesterolemia and Hypertension

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Background: Elevated levels of low density lipoprotein(LDL),serum trigyceride(TG),serum lipoprotein(a)-[Lp(a)] and low levels of high density lipoprotein(HDL) have been documented as leading risk factors for the development of coronary heart disease(CHD) in Indian women.In view of an increment in LDL levels with fish oil supplementation (Suppl'n) ,the effect of a combined suppl'n of fish oil (MEGA-3) with garlic oil (GARLIC PEARLS) on the anthropometric measurements (AM), serum lipid profile(SLP) and blood pressure(BP) in women with hypercholesterolemia and hypertension were studied.
Aims: To determine the effect of fish oil(FO) with garlic oil (GO) suppl'n on the AM like body mass index(BMI) ,waist circumference (WC) and waist:hip ratio(WHR) ,SLP and BP levels of the premenopausal women(Group-I) and postmenopausal women(Group-II) in the test groups and to compare the same with that of the respective control groups (Placebo).
Methods: This study included 60 hypercholesterolemic (>220 mg/dl) and hypertensive (>140/90 mmHg) women,of which, an equal number((n =30) belonged to the premenopausal group(30-45 yrs.) and the postmenopausal group(46-60yrs.). The subjects were further equally sub-divided (n=15) within each group as control group and test group.The study was conducted for a period of 90 days and with a withdrawal period of 30 days (Test groups).The dosage of fish oil was 600mg per day and garlic oil was 500mg per day(Test groups).The biochemical parameters were analyzed using the enzymatic kit methods .
Results: Statistically significant reductions were seen in all the AM, SLP(except HDL) and BP levels of the women in the test groups after 90 days of suppl'n compared to that of the respective control groups.After the withdrawal period,the hypolipidemic and hypotensive effect of FO with GO was sustained in both the test groups.Minor reductions were reported in the AM in both the test groups The systolic and diastolic BP reduced by 14.8% and 14.9% respy in Group-I compared to 20.3% and 15.9% in Group-II. The total cholesterol(TC),LDL,Very low density lipoprotein(VLDL), serum TG,TC:HDLratio, TG:HDL ratio and serum Lp(a) reduced by 25.5%,33.3%,24.8%,24.7%,28.2%27.3% and 30.3%.respy in Group-I compared to 21.8%, 27.7%, 18.6%, 18.3%,22.8%, 17.9% and 14.6% respy in Group-II.
Conclusion: The co-administration of garlic oil with fish oil was more effective than placebo in the management of dyslipidemia and hypertension in women.

Development of an Inactivated Rotavirus Vaccine for the Global Immunization Agenda

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Background: The efficacy of two live oral rotavirus vaccines (ORV), RotaTeq® and Rotarix®, has been demonstrated for children in developed and middle-income countries but remains to be confirmed for children living in resource-poor countries where rotavirus remains a major killer. We have pursued the development of an inactivated rotavirus vaccine (IRV) as a backup strategy for ORVs. IRVs could be more uniformly reliable when used in the challenging populations. IRVs would not be burdened by the threat of intussusception, a known complication of earlier ORVs and could be tested in smaller clinical trials powered for efficacy (ie. <5000 children) rather than safety against intussusception (>60,000) with ORVs. IRVs could be combined with other parenteral EPI vaccines making them easier to administer as part of routine childhood immunizations. Finally, IRV does not replicate so its efficacy should be free from interference from antibodies obtained through cord blood or breast milk, a problem that may inhibit the immune response to ORVs in children.
Methods: We examined the role of serum antibody in protection against rotavirus infection or disease in a primate model or children with acute diarrhea. We assessed the immunogenicity and protective efficacy of our candidate IRV in mice and gnotobiotic piglets.
Results: We have demonstrated that serum IgG is either an effector or a reliable proxy against rotavirus infection and disease in monkeys and children and established the proof of principle that serum antibodies can protect against rotavirus replication and shedding. We have gone on to develop a candidate IRV from human rotavirus strains and a novel method to effectively inactivate rotavirus while maintain the structural integrity of the viral particles. This candidate IRV adjuvanted with alum and administered intramuscularly to mice elicited strong total antibody and neutralizing antibody responses in serum. This IRV further induced solid protection against rotavirus infection in piglets.
Conclusion: IRV could provide an important insurance policy for the global immunization agenda if ORVs fail to provide adequate protection to those children in low income countries at greatest risk of a fatal infection or if problems of intussusception should again arise from ORV immunization.

Cytochrome P450 Inactivation By Pharmaceuticals And Phytochemicals: Therapeutic Relevance

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Background: One of the major clinical concerns is possible drug interactions that can be the result of abrogation of the P450 pathway(s) of metabolism causing toxicity due to elevated exposures of other drugs metabolized by these pathways. When the P450 substrate is catalytically activated to a reactive intermediate, this transient molecule may react with available nucleophilic residues from the enzyme -- thereby resulting in the inactivation of the P450.
Methods: The effects of CYP inactivation on the pharmacokinetics of co-administered drugs or on the inactivator itself depend on complex factors involving the molecular entities, the kinetics of inactivation (K_I, k_{inact}), the partition ratio, the zero-order synthesis rate of new enzyme, multiple pathways of metabolism (competing pathways), the dose or exposure, and specific patient characteristics.
Results: This review summarizes the catalytic efficiencies of many inactivator drugs along with any consequent clinical relevance. The chemical agents described have been ranked for the kinetic efficiency of inactivation and contrasted with the known clinically relevant drug interactions.
Conclusions: This will allow judicious consideration of the many factors that influence the importance of CYP inactivation and their relative contribution to systemic clearance of co-administered drugs. This study allows an improved characterization and dissection of potential physiological interactions with various drugs and nutrients. Knowing more about selective inactivation of cytochrome P450 by common xenobiotics, drugs and phytochemicals allows better understanding of expected interactions with chemotherapeutics and other xenobiotics.

Bioconjugated Cytotoxin Prodrugs and Imaging Agents in Anticancer Research

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Background: The enediynes are a class of potent antitumoral agents isolated from soil bacteria. One of these (Mylotarg®) represents the first monoclonal antibody-cytotoxin conjugate to be approved by the FDA and is currently used for treatment of acute myeloid leukemia (AML). The natural enediynes use a variety of elaborate triggering mechanisms which activate the pharmacophore through cycloaromatization, producing cytotoxic diyl radicals which damage DNA and other cellular and nuclear macromolecules. There has been considerable interest in the preparation of synthetic enediyne prodrugs that can be delivered to specific targets, and we are currently investigating photo-activated variants.
Methods: A family of substituted aryl enediyne templates were synthesized using standard Pd coupling methodology. The building blocks were converted to various PEG conjugates, which allowed subsequent coupling with targeting antibodies via NHS chemistry. The enedynes were also coupled to Au nanoparticles using standard thiolate chemistry, to allow development of heterobifunctional carrier systems.
Results: The PEGylated enediynes and their Mab conjugates undergo rapid photocyclization to produce the cytotoxic radicals on demand. Furthermore, the immunocompetence of the Mab conjugates are preserved, as confirmed by ELISA. Preliminary experiments confirm the selective cytotoxic activity of the Mab conjugates in cellular bioassays.
Conclusions: Readily available aryl enediynes can be prepared and converted to versatile and thermally stable bioconjugates. Subsequent photoactivation of the systems is equally effective in parent and Mab conjugated state, allowing the development of targeted cytotoxins and biochemical reagents. An immediate possibility is the development of heterobifunctional Au nanoparticles which can be selectively loaded with the PEGylated enediyne and Mab's, and can also accomodate imaging tags for animal studies using PET and SPECT.

Short-term, low dose methotrexate for immune tolerance induction

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Background: Antibody responses that develop against protein therapeutics can potentially impact patient safety and therapeutic efficacy. In an effort to control therapy-specific antibody responses, various approved immunosuppressive agents were evaluated for their ability to induce immune tolerance to two protein therapeutics, recombinant human □-galactosidase (rh□-Gal) and recombinant human acid □-glucosidase (rhGAA). These include mycophenolate mofetil, cyclosporine A with azathioprine, CTLA4-Ig fusion protein, rapamycin, cyclophosphamide and methotrexate.
Methods: C57Bl/6 wild-type, Balb/c wild-type, $6^{neo}/6^{neo}$ GAA knock-out mice (GAAKO), □-galactosidase knock-out mice and x-linked immunodeficiency mice were immunized with either rhGAA or rh□-Gal in the presence and absence of the following immunosuppressive agents: mycophenolate mofetil, cyclosporine A with azathioprine, CTLA4-Ig fusion protein, rapamycin, cyclophosphamide and methotrexate. rhGAA-specific and rh□-Gal-specific antibody responses were evaluated by ELISA. The cellular immune response to rhGAA was investigated in C57Bl6 wild-type mice and GAAKO mice using flow cytometry. Immunogenicity studies involved 8-10 animals/group and were repeated three times. Flow cytometry studies involved 5 animals/group and were repeated three times.
Results: The only agent that successfully induced a long-lived reduction in anti-rh□-Gal and anti-rhGAA antibody responses following short-term immune suppression was methotrexate. For example, three weekly courses of 0.5mg/kg of methotrexate could significantly reduce rhGAA-specific antibody responses by 47% through at least eight months of weekly rhGAA treatment in GAA knock-out mice. Flow cytometry-based studies characterizing the cellular immune response to rhGAA helped inform the treatment schedule and effective dose of methotrexate.
Conclusion: Short-term, low dose methotrexate can significantly reduce antibody responses to at least two different protein therapeutics. Currently, low-dose methotrexate is under clinical evaluation as part of an immune tolerance induction regimen for rhGAA in Pompe patients. Low dose methotrexate may hold promise in reducing antibody responses against protein therapeutics in general.

Is Cyclosporine The Magic Bullet In Dermatology?

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The effects of cyclosporine explain the interest in its history. Since the drug of fungal origin with anti-inflammatory properties, low myelotoxicity, and the T-cell immunomodulating effects revolutionized transplantation medicine, it was claimed that serendipity played a significant part in its discovery. Cyclosporine acts primarily during T cell activation by modulating lymphokine production and by reducing the activation and proliferation of T-helper and cytotoxic T cells. These selective effects have been recognized as beneficial in the treatment of dermatologic diseases, and this has particularly revolutionized skin immuno-interfering. The only dermatological indication for cyclosporine approved by the US Food and Drug Administration is psoriasis. The authors discuss excellent therapeutic responses reported in patients with other dermatologic diseases: atopic dermatitis, erosive mucosal lichen planus, Behcet's disease, epidermolysis bullosa acquisita, lichen planus and pyoderma gangrenosum. Cyclosporine A can be safely administered when potential toxicities, dosing (3-5 m/kg/d), and guidelines are known. Also, highly variable results of systemic cyclosporine in the treatment of severe alopecia areata, have been achieved. The authors of this review following the concepts of lower dosages of systemic cyclosporine (2.5 mg/kg/d decreased by 0.5 mg/kg monthly) and the use of low dose prednisone simultaneously (5 mg/d), developed complete long-duration sustained terminal hair re-growth in universal alopecia areata (chances for cure were less than 1%). Thus, in spite of the tremendous progress in immunologic research, practical therapeutic immune-intervention has yet to reach the level of specificity and precision that is needed, i.e. the "immunologic magic bullet". However, cyclosporine is a good candidate.

Discovery & Development of Antineoplastic Magic Bullets from Natural Products

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Background: Most clinically useful anticancer drugs were discovered from natural products as examplied in adriamycin, vinblastine, taxol, and camptothecin, etc. Aims: 1) A practical and stereospecifc syntheis of novel derivatives of deoxoartemisinin and daumone, a kind of pheromone. 2) *in vitro* and *in vivo* testing of antineoplastic activity of the synthesized compounds. 3) Establishment of strucure and activity relationship(SAR) to design and provide an anticancer drug candidate.
Previously, we discovered a novel pheromone that postpones aging in worms from *C. elegans* with a laborious, a large-scale purification and 3-dimensional structural determination.
Methods: This study included synthesis, anticancer activity screening and SAR study including proposed biomechanism of anticancer activity. Most of synthetic efforts have been focused in derivatization at C-12 position of artemisinin resulting acetal-type derivatives. To overcome instability in simulated stomach acidic condition, and recently appearing neurotoxicity of artemisinin and its C-12 acetal derivatives such as arteether, artemether, and artelinic acid, we prepared non-acetral type deoxoartemisinin and its derivatives from artemisinin, a natural product isolated from *A. annua* as potential anticancer drug candidates. Total synthesis of new derivatives of dauer-effect pheromone isolated C. elegance were efficiently achieved.
Results: Non-acetal C-12, 13 derivatives, (+)-deoxoartelinic acid and dimers of (+)-deoxoartemisinin were synthesized either from naturally occuring artemisinic acid or directly from artemisinin via a short and regiospecific process. Some of its novel derivatives show comparable antitumor activities to those of clinically useful drugs.
A stereospecific, 10 step synthesis of pheromone was successfully achieved starting from commercially available rhamnose. Both isolated and synthetic daumone induce the morphological changes that accompany worm hibernation.
The detail of stereospecific synthesis and the antneoplastic activities of (+)-deoxoartemisinin, its C-12 and 13 derivatives, natural plakortolide and novel anticancer derivatives of daumone along with the structure-activity relationship will be presented. In vitro antineoplastic screening of derivatives of deoxoartemisinin and daumone against human cancer cell lins (ovary, lung, brain, colon) showed potential anticancer activities.
Conclusions: 1).The non-acetal type and anticancer derivatives of deoxoartmisinin showed acid stability, and may overcome neurotoxicity.
2). These results may shed light on the discovery of antineoplastic magic bullets derived from natural products and a signaling pathway thought to be similar to those that lead to aging and obesity in humans. 3). Trimers of deoxoartemisinin and daumone against human cancer cell lins showed potential anticancer activities comparable to that of clinically useful anticancer drugs.

Composition Of Essential Oil Of *Nigella sativa (L.)* Seeds And Inhibition Of Human Neutrophil Elastase

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Background: Inhibition of Human Neutrophil Elastase (HNE) activity by essential oil extracted from Nigella sativa (L.) seeds was tested in this study.
Methods: Hydrodistillation method was used to extract essential oil and the yield was found to be 0.4%. Chemical analysis of essential oil was performed by GC-MS. Determination of HNE activity was realized by spectrophotometry method using N-Methoxysuccinyl-Ala-Ala-pro-Val p-Nitro-Anilide as elastase substrate. Essential oil (5.8 mg/mL) was a strong inhibitor to HNE.
Results: Aggregate effects of total essential oil caused 100 % Inhibition. testing the inhibitory effects of major constituents of essential oil on HNE activity by microassays revealing that 5-Isopropyl-2-Methyl Phenol was a strong inhibitor to HNE with a very low IC50 (12 µM).
Conclusions: We conclude that 5-Isopropyl-2-Methyl Phenol is a natural antielastase compound that could be used in the treatment of some pathological cases such as COPD and emphysema

The Importance of Appropriate Animal Models for Evaluating Agents for Proliferative Retinopathy: Failure of Combretastatin to inhibit Diabetes-Like Proliferative Retinopathy in the Galactose-Fed Dog.

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Background: "Magic bullets" for ocular use have primarily focused on the treatment of neovascularization and the prevention of secondary cataracts. Patients with macular degeneration are intravitreally injected with anti-VEGF antibodies to reduce angiogenesis while anti-neoplastic agents are being investigated for the prevention of post-surgical lens cell proliferation. Combretastatin A-4 (CA4P) is a vascular targeting agent that destroys rapidly growing tumor capillaries. In mouse eyes, CA4P also suppresses retinal neovascularization that occurs within days to weeks following laser induced choroidal neovascularization or genetic over expression of retinal VEGF. To determine the clinical potential of CA4P to treat neovascularization in diabetics, we have evaluated CA4P in galactose-fed dogs, an animal model that slowly develops proliferative retinopathy similarly to that observed in diabetics.
Methods: Eight beagles fed 30% galactose diet for 80-104 months and 4 age-matched normal beagles were made aphakic so that retinal changes could be easily doumented. These were divided into 2 groups composed of 4 galactose-fed and 2 control dogs. Each group received CA4P as either sub-Tenon's or intravitreal injections. Six weeks later, all dogs received systemic (IV) injections of CA4P. Changes in neovascularization and blood flow were clinically monitored at 2-week intervals by fluorescein angiography and fundus photography.
Results: CA4P was well-tolerated in the healthy eyes of normal dogs. In galactosemic dogs, CA4P administration was associated with corneal edema and increased intraocular pressure. All galactose-fed dogs demonstrated retinal neovascular lesions. Sub-Tenon's, intravitreal or systemic CA4P administration failed to alter these retinal lesions.
Conclusions: Failure of CA4P to ameliorate neovascularization suggests that chronic, long-term administration is required to destroy the slowly growing retinal endothelial cells. This treatment is unlikely to be clinically useful for reducing neovascularization in diabetics.

Antibody Based Protein-Function Analysis On The Ryanodine Receptor And Troponin Isoforms In *Caenorhabditis Elegans*

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Background: After genome sequence data are accumulated, understanding of the relation between structure and function of a big molecule or isoform proteins is important to solve biological details. Aims: 1) To compare the bacteria produced fusion proteins and a native ryanodine receptor. 2) To develop antibody based protein analysis that can describe a molecular difference in muscle proteins. 3) To propose a tool for comparing closely related proteins from animals.
Methods: We constructed several expression vectors to generate recombinant proteins of the ryanodine receptor, three body-wall and one pharyngeal troponin I isoforms and other muscle components in *Escherichia coli*. Protein overlay assays and Western blot analyses were performed using anti-rabbit antibodies. Tissue specific localization of the protein is also performed to know whether raised antibody can detect *in situ* localization of the isoforms.
Results: Ten region-peptides of the ryanodine receptor of *C. elegans* (CeRyR) containing 5071 amino acid residues were produced in *E. coli*. One of eight anti-rabbit anti-sera enabled detection of CeRyR *in situ* but others didn't. One peptide had Ca^{++}-binding activity. Using peptides and peptide specific-antibodies analysis we proposed the local structure and function of individual domains within a large molecule. Similar approach in other experiments we demonstrated that pharyngeal TNI-4 interacted with only the pharyngeal isoforms of troponin C/T and tropomyosin in *C. elegans*. In contrast, the body wall TNI-2 bound both the body wall and pharyngeal isoforms of these components. As is known in other invertebrates, the N-terminus of troponin I contribute to interactions with troponin C. Full-length troponin I was essential for interactions with tropomyosin isoforms. From these molecular interaction results we can estimate evolutional history of tissue specific TN isoforms of muscle proteins.
Conclusions:
1) Region specific peptides of a big molecule keep a local structure of whole protein. 2) Antibody based analysis on molecular interactions allows us more information than those of sequence alignments.
This experimental approach is powerful to solve biological function of many accumulated protein sequences and is applicable for determining epitope in functional regions

Peculiarities of Blood-Brain-Barrier Penetration of Pralidoxime

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Background: Organophosphate poisoning in agriculture (millions of cases) and the threat of chemical terrorist attacks require intensive research on antidotes. Organophosphates cause overabundance of acetylcholine at all kinds of its receptors in the central nervous system, and the periphery. Antidote treatment of victims is possible, the therapy is known by the acronym "A FLOP" (atropine, fluid, oxygen and pralidoxime). Clinical experiments, however, have been disappointing with any one of the presently available aldoximes. Fast and effective disposition and excretion of the organophosphate – aldoxime conjugates (phosphoryloximes, POXs) may have vital importance as POXs play an essential part of anticholinestrase activity, and POXs may be hydrolyzed with endogenous enzymes to give the original organophosphates.
Kuca et al. synthesized a series of very effective bis-pyridinium aldoximes marked with the letter K- and a serial number. Petroianu et al. examining relative risk of death of rats in pesticide organophosphate poisoning found K-27 and K-48 to be the most effective antidotes. When ability of several aldoximes to reactivate tabun-inhibited acetylcholinesterase was evaluated by Musilek et al. and superiority of K-203 was found.
Methods: Lipophilicity of pyridinium aldoximes was determined using on silica (planar chromatography) and in silico (computer assisted lipophilicity calculations) methods. Distribution/penetration of pyridinium aldoximes were determined using reversed-phase chromatography of blood, CSF (cerebrospinal fluid) and brain samples of rats following intramuscular treatments of the animals. Various brain regions were also dissected.
Results: Both thin-layer chromatography and computer-assisted lipophilicity calculations showed highly polar characteristics of pyridinium aldoximes. Even, pralidoxime and the other N-substituted pyridinium aldoximes do enter the brain and the cerebrospinal fluid. Results indicate that low doses of pralidoxime, K-27, K-48 and K-203 doses (below 10 microM/rat) show higher relative brain and CSF penetration. Considering the various brain parts (frontal cortex, hypothalamus, hippocampus, striatum, etc.) pyridinium aldoximes show an even distribution.
Conclusions: For patients who have been poisoned, infusion with low-concentration pyridinium aldoximes may be preferable to one injection with a high dose.

Novel Immuno - Potency Drugs

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Background:
The protective role of the immune system in health and the correlations of its failures as a path of pathogenesis are well clarified. Based on recent studies the immunologic concern in the field of health has been improving. The occurrence of diseases like viral, bacterial and fungal diseases, auto- immune diseases and cancer in turn denotes to immunological problems as natural and acquired immune system defects. Because of critical need to support the immune system we proposed that there is need to design kinds of novel generations of drugs to get such a goal. We aimed with this strategy to support the immune system in a way through which it potentiates itself to fight against pathogenic factors, regulate, modulate or reverse the defects as needed.
Methods:
Three different drugs prepared from bacterial and lipid extracts (G2, PC & G2F) that could increase Th-1 cells response depending on the methods of experiments including Delayed type Hypersensitivity (DTH), Lymphocyte Transformation Test (LTT) with PPD and rgp63 cocktails. They prepared in cream and injection forms. The clinical trials Phase I, II performed successfully and also III done in regard with some diseases.
Results:
Based on methods of experiments Th-1 response was significant (P<0.01 to P<0.001). Cytogenetic and mutagenetic studies showed no chromosomal abnormalities .Healing of up to 42% ductal adenocarcinoma of breast cancer in laboratory mice with significant increase in survival lives(P=0.000). Control and cure or prevent after surgery of different cancers in volunteers such as: Leukemia, Breast Cancer, Macroadenoma, Prolacinoma of pituitary gland, colorectal cancer, Hepatoma and other types. These drugs controlled and cured (followed up) of 70 % of adult and up to 90 % of children asthmas respectively.
Complete healing of grade 3-5 of diabetic foot ulcers in some volunteers with resistant to therapy wound types. Newly generated foot ulcers in diabetic patients cured within few days.
Conclusion:
We concluded that the formulations mentioned above are optimized compounds of effectiveness with no side effects reported till now. Because of its effectiveness we suppose that it would be considered as a remedy of next decade. It would be applicable in treatment of chronic diseases, cancers and in all fields required the body to get normal by regulating, modulating and reversing the defects.

Successful Treatment Of Methicillin-Resistant Staphylococcus Aureus Meningitis Using Linezolid Without Removal Of Intrathecal Infusion Pump: Three Years Follow-Up

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Abstract:
Infection of an intrathecal pump system is a rare but serious complication and usually leads to the removal of the pump. We report the first case of methicillin-resistant *Staphylococcus aureus* (MRSA) meningitis in a patient with such a pump successfully treated with linezolid without the need for removal of the intrathecal pump. A 77-year old woman with cervical myelopathy underwent implantation of an intrathecal pump system for baclofen administration. Two weeks after the procedure she developed meningitis caused by MRSA as isolated in cerebrospinal fluid (CSF) cultures, blood samples, and serum obtained from the pump pouch. Clinically she presented with meningism,
somnolence, and signs of sepsis. With a combined intravenous antibiotic treatment regimen of vancomycin and rifampicin no clinical improvement occurred. The regimen was then discontinued and linezolid was administered intravenously as monotherapy. Within 3 days clinical and laboratory findings showed significant improvement. After 1 week of linezolid treatment, blood and CSF cultures were sterile. Intravenous treatment was administered for a total of 3 weeks, after which the patient was treated with oral linezolid for 3 months. During 36 months now of follow-up, no signs of infection or any severe adverse events were observed. These results confirm previous reports of the efficacy of linezolid for the treatment of severe infections of the central nervous system caused by multidrug-resistant Gram-positive bacteria, especially postneurosurgical infections.

A Preparation Of NI-Lipid Nanoparticles By Combination Of Roll Mill And High Pressure Homogenization, And Stabilization Of The Nanoparticles By Gel Solidification Method --Aiming At Preparation Methods Of The Nanoparticle Without Using Organic Solvent—

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Background: Nanotechnology has recently been attracting attention, and establishment of pharmaceutical technologies to micronize drug particles is extremely expected. However, almost all methods use organic solvents in the process of preparing nanoparticles. Accumulation of residual solvents in the body and environmental pollution by liquid waste are matters of concern regarding the use of organic solvents. Thus, we try to prepare nanoparticles without using organic solvents. In addition, we investigate a method to maintain the dispersed condition of the nanoparticles by adding gelatin and solidifying the suspension. Freeze-drying allowed reproduction of the nanoparticle condition. However, this procedure is inconvenient for oral administration due to requiring preparation at the time of use. Simple method substituting for freeze-drying method was desired.
Methods: A 30:1000 (weight ratio) of Nifedipine (NI)-phospholipid (PL) mixture prepared by roll milling was dispersed in water and premixed. Subsequently, the premixed suspension was applied to high-pressure homogenization (HPH). Physical characteristics of NI-PL mixtures were analyzed by using powder X-ray diffraction and Fourier-transform infrared spectroscopy. Particle size was measured by using light scattering photometer.
Results: The mean particle size of the NI-PL nanoparticles decreased as the pass number increased, and the size after 40 passes were 55nm, indicating that roll mixing was as effective as ethanol treatment. NI-specific diffraction peaks of powder X-ray diffraction appeared at 8.2, 16.2, 24.4, and 25.9°. The peaks of NI-PL mixtures were present at the position of NI crystals, and no peak shift was induced by interaction with PL, showing that NI mostly remained as crystal in the PL.
Conclusions: 1) The NI-PL nanoparticle suspension prepared by combination of roll mill and HPH, indicating that nanoparticles could be prepared without organic solvents. 2) The mean particle sizes were about 55nm before and 24h after the gelatin solidification, suggesting that gel solidification method is helpful.

Synbiotics: An Important Tool For Preventing Enterocolitis And Promoting Physical Growth In Severely Ill Pediatric Patients –10 Years' Experience In One Institute-

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Background: Pediatric surgical patients often suffer from severe enteritis and it causes sepsis, hepatic failure and malnutrition. In order to disclose the etiology of these complications we investigated the intestinal microbiota of the patients and the effects of probiotics.
Methods: We designed a new synbiotic therapy consisted of *Bifidobacterium breve Yakult*, *Lactobacillus casei Shirota*, and galactooligosaccharides. Our experience was the first clinical trial of synbiotic therapy in the world. We applied synbiotic therapy to more than 50 pediatric patients who had short bowels, intestinal functional disorders, severe respiratory distress, and liver dysfunctions with abnormal intestinal microbiota (therapeutic use of synbiotics). Intestinal microbiota and nutritional states were followed before and after the therapy. Recently we administered our synbiotics to neonatal surgical patients who had not yet acquired intestinal microbiota (prophylactic use of synbiotics).
Results: Almost all patients had a quite abnormal intestinal microbiota; decrease of anaerobic bacteria and increase of pathogenic microorganisms. After starting the therapy many patients acquired the probiotics dominant intestinal microbiota and it was maintained well during through the treatment course. When the patients recovered from the critically ill states, the intrinsic anaerobes increased in the intestine and probiotics suppressed in its number. The nutritional states improved with synbiotic therapy in many patients.
As to prophylactic use of synbiotics, being still preliminary results, it is very effective for severely ill infants to establish anaerobes dominant intestinal microbiota and maintain good nutritional states.
Conclusions: 1, Our synbiotics are safe and effective for pediatric surgical patients with abnormal intestinal microbiota. Probioitcs were well resided in the intestine and prevented enteritis, improved intestinal functions and patients' nutritional states (therapeutic use of synbiotics). 2, Early start of synbiotics for neonatal patients is more effective to acquire normal intestinal microbiota and it is recommended for very severe neonatal surgical petients (prophylactic use of synbiotics).

Anti-Infective Properties And Functions Of Betaine (Trimethylglycine) As An Nutritional Agent

KANBAK G

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Betaine is found in most microorganisms, plants, and marine animals. Its main physiologic functions are as an organic osmolyte to protect cells under stress and as a source of methyl groups needed for many biochemical pathways. Betaine is also found naturally in many foods and is most highly concentrated in beets, spinach, grain, and shellfish. Strains of enterobacteria that cause urinary tract infections are able to grow in urine with high tonicity. The betaine analogues have antibacterial effect against E. coli strains, but only in presence of an osmotic stress. Analogues of gamma-butyrobetaine appear to be prospective drugs for the treatment of circulatory complications of sepsis. Betaine is used by cells to defend against changes in osmolarity. It is suggested that there are relationships among betaine, osmolarity and coccidiosis. The chemotaxis of monocytes toward chemotactic factors released by heterophils was increased by betaine. Increased chemotaxis of monocytes and NO release by macrophages may explain the decreased intestinal pathology but increased leukocyte numbers that were observed when betaine was fed during a Cocci infection. Urine has long been known to inhibit the activity of aminoglycosides against urinary tract pathogens. Glycme betaine which is present in urine confers resistance against high osmolarity to Gram-negative organisms. The betaines in urine permit the expression of increased resistance to aminoglycosides in concentrated urine. Hepatitis C virus (HCV) infection is an important cause of chronic liver disease. Standard therapy, pegylated interferon alpha (pegIFNalpha) combined with ribavirin, results in a sustained response rate in approximately half of patients. It has been shown that treating cells with S-adenosyl-L-methionine (AdoMet) and betaine could restore STAT1 methylation and improve IFNalpha signaling. Furthermore, the antiviral effect of IFNalpha in cell culture could be significantly enhanced by the addition of AdoMet and betaine.. In our study, we investigated the protective effect betaine and prednisolone on the level of nitric oxide in sepsis. As a result, according to hematologic and biochemical assessments, we concluded that betaine administration without prednisolone is more useful and effective in decreasing susceptility to sepsis.Our data in our laboratory and recent studies in literatures showed that betaine (trymethyglycine) and betaine analogues have anti-infective peroperties

Epitopic Peptides With Low-Similarity To The Host Proteome. Towards The Magic Bullets.

KANDUC D

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A robust set of experimental data suggests that low level of sequence similarity to the host proteome modulates the B cell epitope pool in the humoral immune response. The data appear to be widely supported by structured meta-analyses of scientific literature. Theoretically, proteomic similarity analyses might elucidate the regulatory mechanisms/factors that dictate peptide immunogenicity assessment. From the clinical point of view, low-similarity peptides may have a strong repercussion on the rational development of peptide-based treatments in cancer, infection and autoimmunity. *De facto*, the most attractive feature of the similarity concept is that it appears a guarantee of highest specificity and lowest cross-reactivity in designing effective, safe and theoretically infallible immuno-therapeutical tools. The Ehrlichian idea of therapeutic agents equipped with high affinity to the causative agent and efficacy in concentrations harmless for the patient, appears feasible. The Ehrlichian *therapia magna sterilisans* might be at hand.

Ames Test And Gene Expression Analysis For Polyhydroxybutyrate – A Biomaterial

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Background: It is imperative to test new materials for mutagenic or carcinogenic properties before they are approved for medical use. Polyhydroxybutyrate (PHB), is finding many useful applications as an implant material due to its biocompatibility and resorbability.
Aims: 1) To determine the mutagenicity of a locally produced PHB using *Salmonella* mutagenicity test. 2) To determine whether PHB alters the expression of p53 and c-myc proto-oncogenes and bcl-xl and bcl-xs anti-apoptotic genes in the human fibroblast cell line.
Methods: Different concentrations of PHB (5, 2.5, 1.25, 0.625 and 0.3125 mg/plate) were incubated with special genotypic variants of *Salmonella* strains (TA1535, TA1537, TA1538, TA98 and TA100) carrying mutations in several genes both with and without metabolic activation system (S9) and the test was assessed based on the number of revertant colonies. Concurrently, both negative (sterile distilled water) and positive controls (sodium azide, 9-aminoaridine hydrochloride monohydrate, 4-nitro-O-phenylenediamine and 2-aminoanthracene) were used. For the gene expression analysis, fibroblast cell line (CCL-171) designated as MRC-5 was used. The cells were treated with five doses of PHB (0.3125, 0.625, 1.25, 2.5 and 5 mg/ml) and incubated for 1, 12, 24 and 48 hours at 37ºC in a CO_2 incubator. The total RNA was isolated and reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out for β-actin, p53, c-myc, bcl-xl and bcl-xs genes. The amplified products were run on an agarose gel and then quantified based on the intensity of bands of the different genes.
Results: The average number of revertant colonies per plate treated with PHB was less than double as compared to that of negative control. Also, the PHB did not show over or under expression of the genes studied.
Conclusions: 1) The *Salmonella* mutagenicity test indicated that the locally produced PHB is non-mutagenic on the variants of *Salmonella* strains TA1535, TA1537, TA1538, TA98 and TA100. 2) PHB does not alter the expression of the proto-oncogenes (p53 and c-myc) and anti-apoptotic genes (bcl-xl and bcl-xs) in this study under the present test conditions.

Alpha2-Antiplasmin Is A Critical Regulator On Bleomycin-Induced Fibrosis

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Background: Fibrotic disease occurs in most tissues. It has been known that the transforming growth factor (TGF)-□ and connective tissue growth factor (CTGF) are the main regulators of fibrosis, and TGF-□□□□□□□□ the expression of CTGF. The fibrinolytic system is considered to play an important role in the degradation of extracellular matrices (ECM). However, the detailed mechanism how this system affects fibrosis remains unclear.
Methods: To verify the effect of the α2-antiplasmin (α2AP), which is a potent and specific plasmin inhibitor on fibrosis, we showed a histological examination on bleomycin-induced dermal fibrosis in $\alpha2AP^{+/+}$ mice and $\alpha2AP^{-/-}$ mice. In addition, we examined that the level of mRNA on bleomycin-induced dermal fibrosis in $\alpha2AP^{+/+}$ mice and $\alpha2AP^{-/-}$ mice by using RT-PCR. Moreover, to obtain genetic evidence that α2AP functions, we examined the levels of mRNA and protein in the fibroblasts from both $\alpha2AP^{-/-}$ and $\alpha2AP^{+/+}$ mice by using RT-PCR and ELISA.
Results: We found that the absence of α2AP attenuated bleomycin-induced TGF-β synthesis and fibrosis. In addition, the production of TGF-□ from the explanted fibroblasts of $\alpha2AP^{-/-}$ mice decreased dramatically compared to that in $\alpha2AP^{+/+}$ mice, and α2AP specifically induced the production of TGF-β in fibroblasts. Moreover, we found that CTGF induced the expression of α2AP.
Conclusion: α2AP is involved in the production of TGF-β, and CTGF induces the expression of α2AP. This study suggests that α2AP plays a crucial role on the progression of fibrosis. Our findings may hopefully provide new insight into this field which could eventually lead to the development of new clinical therapies for the prevention of fibrosis.

In Vitro Preclinical Studies For A Rational Design Of Cancer Chemotherapy Combinations

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Background: Combination chemotherapy has been used for the treatment of cancer, based upon theoretical advantages and on proven clinical efficacy. In vitro drug combination studies play an important role in designing and optimising combination protocols. However, the design and analysis of preclinical studies to assess the cytotoxic effects in combinations is complex, and there is no consensus as to the best method, since dose-response curves of anticancer agents are variable. Even the same data are used, the results are often different, depending on the evaluation method used.
Methods: A variety of cancer cell lines were used for drug combination studies. Cells were exposed to two drugs simultaneously (and sequentially). Cell growth inhibition was determined using MTT assay. We used the isobologram method (Steel and Peckham) for studying the cytotoxic effects of drug combinations, since it can cope with a variety of doseresponse curves of anticancer agents. This method is generally stricter for synergism and antagonism than other methods.
Results and Conclusions: 1) Therapeutic benefit was highly dependent on the combination and schedule of administration. 2) The anticancer agents which it was easy to use together (additive or synergistic effects in simultaneous exposure) included alkylating agents, anthracyclines, cisplatin, etoposide, imatinib, irinotecan, and antimetabolites (excluding antifolates). 3) The simultaneous administration of methotrexate and all other agents studied produced antagonistic effects, while methotrexate first followed by most agents produced synergistic effects. 4) Similar tendency was observed in pemetrexate in combination with cisplatin, gemcitabine, irinotecan, or paclitaxel. 5) Paclitaxel had a tendency to act subadditively when administered simultaneously but additively when administered earlier. 6) These findings are useful for designing clinical trials of combination chemotherapy.

Differential Targeting Of Immune Response After Antigen Encounter At Different Mucosal Sites – A Tool For Vaccine Development

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Antigen encounter results in activation of specific lymphocytes, which then leave the site of antigen encounter and enter the blood as plasmablasts. These cells are found only temporarily in the circulation while on their way to home to tissues, where they settle down as the final end-stage B cells, plasma cells. While representing only 1% of all circulating B cells, plasmablasts are the only active effector B cells in the circulation, often identified as cells producing specific antibodies against the recently encountered antigen. Plasmablasts express homing receptors (HR) and chemokine receptors (CCR) that allow them to leave the circulation through the endothelium and enter the tissues. Even if blood takes the cells everywhere in the body, plasmablasts can only enter those tissues where the ligands for their HR and CCR are found. The tissue-specificity of homing results from a differential expression of chemokines and ligands for HR between the tissues. The selection of HR and CCR is imprinted on the activated lymphocytes already at the original site of antigen encounter by dendritic cells. Investigation of homing-associated molecules, the homing profile, on plasmablasts provides information on the targeting of the immune response in the body.
Almost all pathogens are encountered at mucosal surfaces. These portals of entry are guarded by the local mucosal immune system. Mucosal immunization is the most effective way to induce mucosal immune responses. The different mucosal sites in the body are regarded to be interconnected with one another via circulating lymphocytes thus constituting a sc. Common Mucosal Immune System (CMIS). Antigen encounter at one mucosal site can elicit an immune response at a distant mucosal site. However, lymphocytes don't migrate equally to all mucosal sites, but there is compartmentalization within the CMIS. Our results show that in humans, antigen encounter in the intestine, urinary, genital, upper and lower respiratory tract each label the circulating plasmablasts with a characteristic homing profile. As the targeting of the immune response depends on the site of antigen encounter, vaccines should be administered to sites from where the immune response is most effectively targeted to that particular site (intestine, genitourinary, respiratory tract), where the immune protection is desired the most.

Infection Site Concentration Of Metronidazole (MTZ) And Meropenem (MER) In Patients With Septic Shock (SS)

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Background: Sepsis is a frequent syndrome and cause of mortality in modern intensive care. The inadequate concentration of antimicrobial drugs in site of infection might be one of the therapeutic failure reasons. Microdialysis (MD) is a sampling technique of tissue fluid, allowing protein-free fluid sampling from different tissues. The main purpose of the present work was to elucidate distribution of MTZ and MERO in clinical conditions such as perioperative prophylaxis, SS and severe peritonitis.
Methods: 18 patients were studied: 6 female patients receiving MTZ in perioperative period, 6 male patients in intensive care unit (ICU) receiving MTZ for the treatment of anaerobic infection and 6 patients in ICU receiving MERO for the treatment of severe peritonitis associated with SS. Both drugs were given intravenously. MTZ sampled from plasma (PL) and muscle tissue (MT) using MD and MERO from PL and peritoneal cavity (PC) using MD. Samples were taken at predetermined time-points before and after administration of drugs. Drugs' concentration were measure using HPLC with ultraviolet detection (MTZ) and LC-MS/MS (MERO). Pharmacokinetic-pharmacodynamic (PK/PD) modeling were also performed: *in vitro* with MTZ and using mathematical modeling with MERO.
Results: MTZ mean max concentration (Cmax) in surgical patients and patients with SS was 16.5 and 11.4 mg/L in PL and 7.8 and 8.2 mg/L in MT, respectively. Time over minimal inhibitory concentration (T>MIC) was 23.3 and 36.2 hours for MT of surgical and septic patients, respectively. The ratio of Cmax/MIC was also higher in septic patients being 31.1 and 32.7, respectively. *In vitro* PK/PD modelling using MT concentration from patients with SS showed that time for kill 99.9% of inoculum is between 1 and 3.5 hours after exposure. MERO mean Cmax in PL and PC was 86.1 and 36.8 mg/L, respectively. T>MIC was at least 87% of interdosing interval for MIC 4 mg/L in both PL and PC and was 55 and 43% for MIC 16 mg/L in PL and PC, respectively.
Conclusions: 1) Distribution of MTZ is not significantly different in healthy patients and SS patients. 2) MTZ demonstrates excellent antimicrobial killing 3) Concentration of MERO in PC in case of severe peritonitis is high enough to produce antimicrobial killing.

Authors' disclosure statement: Concentration of MERO in microdialysates was determined by Lefevre S., Marchand S and Couet W, University of Poitiers, France. The PK/PD analysis for MERO was performed by prof. Sawchuk RJ, University of Minnesota, USA and prof. Couet W, University of Poitier, France

Toxic Effect Of Homocysteine On Nervous And Immune System

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Homocysteine (HC) is a risk factor of neurodegenerative and cardiovascular diseases. In terms of effect of HC and homocysteic acid (HCA, the product of spontaneous oxidation of HC) on NMDA-receptors (Carpenter et al, 1994) toxic effect of HC and HCA on NMDA-receptors on neuronal and immune competent cells was studied. These compounds were found to activate NMDA-receptors in a dose dependent manner (0,1-1 mM) resulting in calcium and reactive oxygen species signal and subsequent activation of MAP kinase. Over-loading of NMDA-receptors with these ligands induces injury process in both neurons and lymphocytes resulting in apoptosis at lower concentrations and necrosis at higher concentrations. Thus hyperhomocysteinemia induces death of the neuronal and immune competent cells resulting in massive exhaustion of both systems. Prenatal hyperhomocysteinemia induced by over-loading of pregnant rats with dietary methionine (1 g per kg body weight) resulted in modification of properties of NMDA-receptors and memory and behavioral deficiency. Treatment of these animals with carnosine, natural neuromodulator and antioxidant preserved the pups against toxic effect of hyperhomocysteinemia preventing loss of body weight and memory deficiency. We have concluded that carnosine may protect metabolic function of neuronal and immune systems against systemic oxidative stress induced by HC.

Valproate (VPA): Unrecognized Value From An Old Molecule

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In Grenoble, in 1963, H. Meunier and his staff were investigating molecules with a ring structure containing a nitrogen atom to suppress epileptic seizure activity in rabbits and mice. They noticed, however, that it was actually the *solvent* of the various nitrogen-containing compounds, a simple dipropyl-acid, that had protective power against cardiazol-evoked epileptic seizures. Subsequently, trials were performed with this solvent, dipropylacetic acid (valproic acid [VPA] or sodium valproate), in adult patients with generalized seizures at epilepsy and psychiatry centers. VPA's great efficacy and safety in epilepsy patients with untreatable seizures prompted European regulatory agency approval as an anti-epileptic drug (AED) in 1967. The discovery of VPA led to a change in concept of working mechanisms for AEDs in general (an increase of the inhibitory neurotransmitter GABA). This conceptualization of the mechanism of action led to the development of new AEDs, e.g., vigabatrine, tiagabine and gabapentin.
Other indications were sought for VPA as early as 1964 by Lebreton.
VPA failed in an anxiety/stress animal model, however, it was noted that 5-10 minutes after intravenous administration of 200 mg/kg VPA in mice, activity and explorative behavior were diminished, indicating a potential role in the treatment of psychiatric disorders. It took until the 1990's before VPA was officially recognized to be effective not only in all types of epilepsy, but also in acute mania (US FDA-approved in 1995) and migraine (US FDA-approved in 1996). At present, VPA is a candidate drug leading to interesting changes in the concepts about working mechanisms of drugs and underlying pathology of the various brain diseases and its interconnections. Recent research demonstrates this unification hypothesis: i. *peak* plasma VPA concentrations are not more efficacious than continuous intravenous VPA infusion; ii.VPA has proven *sustained efficacy* (median = 4 years, up to 12 years) in photosensitive epilepsy patients; iii. a close link exists between migraine, occipito-temporal lobe epilepsy and photosensitivity. The success of VPA therapy clinically has inspired others to search for other neuro-active drugs that can act as a "magic bullet" working across a spectrum of neuro-psychiatric diseases.

DNA Conformation in Complexes with Coordination Compounds

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Background: DNA is the main target for platinum-based chemotherapy drugs used to treat various types of cancers. Cisplatin was the first member of this class, which now also includes carboplatin and oxaliplatin. Platinum compounds interact with DNA in cells, prevent its replication and disrupt repair.The overcoming of the problem of high toxicity of antineoplastic agents is a main challenge in the field of cancer research. The development of drug resistance is the other obstruction that limits the efficacy of the majority of chemotherapeutic agents. The extensive research has been directed toward the seeking for new coordination compounds with the remarkably low toxic side effects compared to the utilized platinum-based drugs. Wide range of new perspective compounds demands an effective and simple method for the preliminary selection of drugs. DNA solution can be corresponded as a model system for the tentative testing of new chemicals.
Material and Methods: Calf thymus DNA (Sigma) MM and plasmid DNA pFL 44 / EcoRI in linear and circular form were used. The set of experimental methods (circular dichroism, UV spectrophotometry, dynamic light scattering, electrophoresis, viscosity, flow birefringence, atomic force microscopy, NanoScope 4a, Veeco) ensures the information about DNA secondary and tertiary structure during interaction with coordination compounds.
Results: DNA conformation in complexes with coordination compounds of Pd(II), Co(III), Ru(III), binuclear compounds of Pt(II) and Pt (IV) with different ligands was regarded. DNA persistence length, double-stranded structure, electrophoretic mobility, volume and shape of molecular coil were examined. It is known that complex ions can interact with DNA via strong covalent binding, van der Waals forces, hydrogen bonding or electrostatic attraction. The similarity and difference between the interactions of coordination compounds and metal ions with DNA are analyzed. The influence of pH and solution ionic strength on DNA interaction with complex ions was investigated. The consistent influence of platinum drugs and gamma irradiation on DNA structure is explored. The possibility of formation of gene vectors with coordination compounds is examined.
Conclusions: The integrated approach to the analysis of DNA conformation in complexes with coordination compounds in a solution can provide the effective tentative testing of new drugs.The investigation of DNA interaction with new compounds in a solution and the comparison of experimental results with data obtained for cis-DDP, non active trans-DDP and other biological active compounds can provide the information about the molecular mechanism of anticancer activity.

Factors That Influence The Prevalence Of Drug-Drug Interactions Between Antiretroviral Drugs Prescribed To Patients Of Different Age Groups In A Section Of Private Healthcare Sector In South Africa

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Background: Drug-drug interactions (DDIs) are often a serious complication due to taking multiple medications and account for 3% to 5% of all in-hospital medication errors (Leape *et al*., 1995). DDIs are of particular concern in HIV/AIDs patients receiving highly active antiretroviral therapy (HAART), particularly certain protease inhibitors (PIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs), for they interact with other antiretrovirals (ARVs). This is due their metabolism through the cytochrome P450 (CYP450) system. AIMS: 1) To evaluate factors that could influence the prevalence of DDIs between ARVs prescribed in different age groups. 2) To determine whether ARVs were prescribed according to the recommended prescribing guidelines in South Africa.
Methods: This was a quantitative, retrospective drug utilisation study performed on 49 995 (N = 19 860 679) and 81 096 (N = 21 473 074) ARV prescriptions that were prescribed to 43 547 (N = 5 433 440) and 70 719 (N = 5 728 371) HIV patients for 2005 and 2006 and claimed through a medicines claims database. Possible DDIs between ARVs were identified according to Tatro (2005).
Results: Of the 5 433 440 and 5 728 371 patients reviewed for both years, 0.80%; 1.23% was HIV positive patients, of whom 43.26%; 57.06% were males and 56.74%; 42.94% were females for 2005 and 2006 respectively. A total of 49 995; 81 096 ARV prescriptions claimed, of which 4.49; 4.07% had one item, 43.75; 43.52% two, 43.86; 49.56% three, 0.07%; 2.78% four and 0.07%; 0.06% had more than four items on the prescription. Of 811 DDIs identified for 2005, 33.54% were for two drug items, 61.90% three, 2.10% four and 2.46% had more than four items. For 2006, 1115 DDIs were identified, of which 59.64% were for 2 drug items, 27.71% three, 11.12% four and 1.52% had more than 4 items. DDIs identified in different age-groups for 2005 and 2006 were: 5.65% (2006) for patients ≤ 12 years, 74.60%; 58.75% for patients > 19 years and ≤ 45 years; 23.55%; 27.35% for patients > 45 years and ≤ 59 years; and 1.85%; 8.25% for patients ≥ 59 years. The most important interactions were identified between combinations of: Kaletra® (Lopinavir 133.3mg/Ritonavir 33.3mg) and Stocrin® (Efavirenz 600mg) at daily doses of 799.8mg/198mg and 600mg respectively, followed by Crixivan® (Indinavir 400mg) and Norvir® (Ritonavir 100mg) at daily doses of 1600mg and 200mg; and Kaletra® (Lopinavir 133mg/Ritonavir 33.3mg) and Nevirapine (Viramune® 200mg) at 1066.4mg/264.7mg and 400mg daily doses. All the interactions were of clinical significance level 2 (moderate effects), causing deterioration of a patient's clinical status. **Conclusions**: These results demonstrate that the non-adherence of the recommended prescribing ARV drug combinations, and daily doses prescribed in different age groups could influence the prevalence of DDIs, therefore a need for more interprofessional education on the prescribing protocols for ARVs.

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EMY162 Protein As A Vaccine Candidate To Reduce Level Of Alveolar Hydatid Disease

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Background: *Echinococcus multilocularis* is a cestode parasite. The life cycle of *E. multilocularis* generally occurs in foxes and rodents as intermediate hosts. Humans can be infected by accidental ingestion of the parasite eggs from an infected fox, or occasionally from infected dogs or cats. Infection in humans causes alveolar hydatid disease. The disease is a significant public health problem. We attempted to clone cDNA of secretory proteins involved in immune defense systems in order to use them in the control of alveolar hydatid disease.
Method: *E. multilocularis* (Nemuro strain) were obtained from a dog-cotton rat life cycle maintained at the Hokkaido Institute of Public Health. Immature adult worms were collected on day 20 post-infection from dog an experimentally infected with *E. multilocularis* protoscoleces. Total RNAs from the adult worms were isolated. A cDNA library based on mRNA from adult worms of *E. multilocularis* was constructed. One of the cDNA clone, *em*Y162, was isolated from this cDNA library. Recombinant emY162 was subcloned, and then recombinant antigen EMY162 was administrated to mice with Freud's complete adjuvant. Antibody production was assayed by immunoblot analysis. Immunobiological reactivity was analyzed by Western blot. After the final immunization by the recombinant antigens, parasite eggs were administrated orally for vaccine trial of the recombinant EMY162. The number of alveolar cyst in each mouse was counted.
Results: The putative protein from *em*Y162 cDNA consists of 153 amino acids and has a predicted molecular weight of 17.0 kDa. The amino acid sequences of EMY162 are predicted to have single fibronectin type III-like domain. The *em*Y162 is expressed in all four stages (protoscoleces, cultured metacestodes, immature adult worms and mature adult worms). When immunity to recombinant EMY162 was examined, strong IgG immune responses were detected in Western blots. The recombinant EMY162 antigen-specific antibody response showed a polarization toward IgG2 subclass. In addition, the recombinant EMY162 induced a significant level of host-protection (74.3%) in experimental infection with *E. multilocularis* eggs in mice, and showed significant reactivity to the sera from alveolar echinococcosis patients.
Conclusion: 1) The EMY162 protein could target both mucosal and systemic immunity in dogs and humans. 2) The EMY162 protein will help the development of both protection against and diagnosis of alveolar hydatid disease.

Ca^{2+}-Signal Transducing System From The Endoplasmic Reticulum To Mitochondria Involved In The Caffeine-Inducible ATP Transport

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Adenocine triphosphate (ATP) is released as an autocrine/paracrine signal from a variety of cells. The present study was designed to clarfy the Ca^{2+}-signal pathway involved in the caffeine-inducible transport of ATP from cultured vas deferens smooth muscle cells. The transport of ATP induced by caffeine (3 mM) was almost completely inhibited by ryanodine and tetracaine, but not by 2-APB, thus being mediated by ryanodine receptor (RyR). The expression of messenger RNA from only RyR-2 was detected in the cells. Furthermore, the induced transport was attenuated by mitochondrial inhibitors, rotenone and oligomycin and by Cl^- channel blockers, niflumic acid and NPPB. Increase in Ca^{2+}-signals with fluo -4 and rhod-2 caused by caffeine were reduced by tetracaine and oligomycin plus CCCP, respectively. A close spatial relation between the endoplasmic reticulum (ER) and mitochondria was electromicroscopically observed in cells, supporting the existence of a Ca^{2+}-signaling bridge on both the organelli. These results suggest that caffeine stimulates ryanodine receptor (RyR-2) and facilitates a Ca^{2+}-signal transducing system from ER to mitochondria, and then, the signal appears to accelerate the ATP synthesis in mitochondria. In addition, the mitochondrial event may lead further cell signaling to the cell membrane and activates Cl^- channels, resulting in the extracellular transport of cytosolic ATP. In the study with MDCK cells, we also provided evidence that such a Ca^{2+}-signaling pathway from ER to mitochondria mediates the transport of ATP induced by adenosine.

Structure Based Development Of Selective Inhibitors For Individual Cathepsins And Their Medical Applications For Therapeutic Purposes

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Background: Cathepsins (Cath.s) are cysteine protease in lysosomes. Eleven kinds of cath.s are registered in human genome. Cath.s play an essential role in protein catabolism. Since each cath. has a different cleavage-bond, different cath.s produce different product from the same protein. Abnormal expressions of cath.s induce special diseases, therefore the specific cath. Inhibitors are useful for pathogenesis and also for the therapy.
Methods: The study of cath. Inhibitors has been started from the discovery of natural inhibitors from bacteria. One is aliphatic aldehyde derivatives to bind with SH-of cath; leupeptin and antipin by Umezawa. The other is derivatives of epoxysuccinate (ES), E-64 by Katunuma and Hanada. However, they inhibited all cath. family. Using these frame inhibitors, we developed the specific inhibitors for individual cath.s, based on their different tertiary structures of their substrate binding pockets using their X-ray crystallography.
Results: 1) Specific cath. Inhibitor design. (a) As the ES derivatives. Cath.B specific inhibitors having-Ile-Pro at the C-terminus; CA-074, and also cath. L specific inhibitors; CLIK-148. (b) As the aliphatic aldehyde derivatives, cath.S specificity inhibitor, CLIK-60. (c) As the pyridoxal derivatives for cath.K, CLIK-164. These inhibitors showed specific inhibition for special cath.s at the 10^{-6} -10^{-7} M level, *in vitro* and *in vivo*. 2) Medical applications. (a) Osteoporosis and bone metastasis of cancer were protected by cath.L or K inhibitor, CLIK-148 or CLIK-164. (b) Antigens are processed by various cath.s. T1-type and T2-type expressions were switched by antigen processing by different cath.s. (c) MHC-Class II was activated by invariant chain degradation by cath.S. (d) In auto-immune Sjögren's disease, the auto-antigen “Hodrin” was processed by cath.S, CLIK-60 suppressed the Sjögren's syndromes in the model mice.
Conclusions: 1) Specific cath. inhibitors were designed and developed. 2) Osteoporosis and bone metastasis of cancer were suppressed by cath.L inhibitor. 3) Antigen processing and presentation were regulated by these inhibitors. 4) Suppression of autoantigen processing in Sjögren D. by CLIK-60 and type-1 Diabetes by CLIK-148.

Melatonin-A Possible Magic Bullet In Reducing Hypoxic Brain Injury

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Melatonin, a neurohormone synthesized and secreted by the pineal gland is reported to have antioxidant, immunoregulatory and neuroprotective actions. Production of melatonin is regulated by light and darkness, light decreasing and darkness increasing it. Its production is also known to decline in old age and under hypoxic-ischemic conditions. Melatonin is considered the body’s chronological pacemaker and has a wide array of useful applications. It has been used in the treatment of sleep disorders, especially those associated with circadian dysrhythmicity, and is also reported to have neuroprotective effects in many central nervous system (CNS) conditions such as amyotrophic lateral sclerosis, Parkinson’s disease, Alzheimer’s disease, ischemic injury, neuropsychiatric disorders and head injury. Results from our laboratory have shown that it affords protection to the blood- brain and blood-retinal barriers in hypoxic conditions by suppressing the production of vascular endothelial growth factor and nitric oxide which are known to increase vascular permeability. Protective effects of melatonin against hypoxic damage have also been demonstrated in newborn experimental animals where it suppressed damage in many parts of the brain such as the hippocampus and choroid plexus in lateral ventricles. Along with this, exogenous administration of melatonin in newborn animals has been shown to be effective in enhancing the surface receptors and antigens on the macrophages/microglia in the CNS supporting its immunoregulatory actions. Keeping these beneficial effects in view, melatonin merits consideration as a potential ”magic bullet” for mitigating brain damage in hypoxic-ischemic injuries.

This study was supported by a research grant (R-181-000-098-112) from the National University of Singapore.

Cardiac Side Effects Of Psychotrop (Antidepressant, Antipsychotic) Drugs

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Background: The most frequent cardiac side effects of psychotrop drugs (antidepressants, antipsychotics) are brady- or, tachycardia, ECG alterations (prolongation of QRS, QT interval), AV-block, ventricular arrhythmias (tachycardia, torsades de pointes, TdP) and sudden death.
Aims and methods: To attempt to find relations between clinical data and the electrophysiological effects of antidepressants (fluoxetine, citalopram), antipsychotics (risperidone) obtained in isolated guinea-pig ventricular muscles and canine ventricular myocytes using the conventional microelectrode and whole cell clamp technique.
Results: Fluoxetine (**F**) (0.5-50 μM) and citalopram (**C**) (10-100 μM) exhibited depressant effects on contraction and both on Ca^{2+} and Na^+- dependent electrophysiological parameters of cardiac preparations and on cardiac Ca^{2+} current, without modifying the K^+ currents. Risperidone (R) (0.1-10 μM) caused a concentration-dependent lengthening of action potential duration (APD) in both preparations and it blocked concentration-dependently the rapid component of the delayed rectifier K^+ current (I_{Kr}) The other K^+ currents (I_{K1} and I_{to}) and Na^+ current were not significantly modified. **Conclusion**: the inhibition of cardiac Ca^{2+} and Na^+ currents by **F** and **C** , moreover the depression of I_{Kr} current by **R** may explain the cardiac side-effects observed occasionally with these drugs. Our results suggest that the new generation of antidepressants (fluoxetine, citalopram) and antipsychotics (risperidone) may have also antiarrhythmic, as well as proarrhythmic properties. Therefore, clinicians should be more vigilant about these potential adverse reactions and ECG control may be suggested during therapy, especially in patients with cardiovasular disorders.

Molecular Epidemiology of Quinolone Resistant *Salmonella* Typhi: South Africa 2003-2007

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Background: Fluoroquinolones have become the mainstay of treatment for typhoid fever in many countries, including South Africa. Resistance to the quinolones antibiotics results in treatment failures and quinolone resistance in South African strains of *Salmonella enterica* serotype Typhi is well documented.
Methods: The molecular mechanism for quinolone resistance in *Salmonella* Typhi from South African clinical isolates received by the Enteric Diseases Reference Unit of the National Institute for Communicable Diseases for the period 2003-2005 was determined using pulsed-field gel electrophoresis (PFGE), multiple-locus variable-number tandem-repeats analysis (MLVA), PCR and sequencing of the quinolone resistant determining region (QRDR) genes gyrA, gyrB, parC and parE, as well as plasmid-mediated quinolone resistance determinants (PMQD) QnrA, QnrB, and QnrS.
Results: PFGE showed 2 major clusters (90%) among the 20 quinolone resistance isolates. MLVA was more sensitive and grouped these same 20 isolates into 11 MLVA types, with the majority (8/20) grouped as MVLA type-16 with the other types differing by 1 allele, either TR1 or TR2. Among the 8 related and non-related PFGE isolates screened for mutations in the QRDR region 2/8 isolates had mutations in gyrA, parC and parE; 2/8 isolates had mutations in parE; 1/8 isolate had a mutation in gyrA and parE; 1/8 isolate had mutations in gyrA, gyrB, and parE, and 2/8 isolates exhibited no mutations in their QRDR regions. PCR screening for as PMQD were all negative.
Conclusions: Although these results seem counter-intuitive to previously published work, the molecular mechanism of quinolone resistance for these isolates may not be attributed to a single mechanism but may be the result of a combination of mechanisms.

The impact of highly active antiretroviral therapy on cytomegalovirus retinitis: triumphs and future challenges

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Background: Cytomegalovirus (CMV) is a common opportunistic infection in individuals with AIDS, with CMV retinitis representing a significant portion of end-organ disease in these patients. Before the development of highly active antiretroviral therapy (HAART), nearly one-third of people with AIDS developed CMV retinitis during their lifetime. Although effective therapies for CMV infection had been developed, treatment was often life-long due to persistent immune deficiency. Even with chronic suppressive maintenance therapy, disease relapse was nearly universal, and development of drug resistance was not uncommon. Aims: 1) To evaluate the impact of HAART on the course and complications of CMV retinitis 2) To identify continued challenges in thetreatment of CMV retinitis and propose further avenues for investigation.
Methods: This study reviewed available evidence in the medical literature concerning the treatments and outcomes of CMV retinitis with particular attention to the impact of HAART and to data derived from the Longitudinal Study of the Ocular Complications of AIDS.
Results/Conclusions: The widespread use of HAART has reduced the incidence and complications of CMV retinitis in patients with HIV infection. With sustained immune recovery, discontinuation of anti-CMV therapy has been possible in many patients. Still, immune recovery does not guarantee protection from recurrent disease. CMV retinitis and uveitis associated with immune recovery remain causes of vision loss in this population. Areas such as genetic susceptibility to CMV retinitis and the development of long-term drugs and drug delivery vehicles apprpriate for developing countries offer further avenues of investigation.

Political Economy and Societal Consequences of Methamphetamine Epidemic in the United States

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The world, beleaguered by a multitude of illnesses and syndromes, anxiously looks to pharmaceutical companies for the creation and distribution of substances that will ease pain, discomfort, and even reverse degeneration. However, a glance through pharmaceutical history tells us that a drug can be both a welcomed panacea and a despised scourge, a literal _magic bullet._ Methamphetamine (meth) is one such substance. A derivative of amphetamine, which was first synthesized in 1887, methamphetamine has roots in 1940s Japan. It mirrors the body_s natural adrenaline surge, and has been a persistent and pervasive worldwide problem, insidiously affecting national fabrics and political economies, and creating widespread societal consequences such as deterioration of public health, destruction of families, domestic violence, robbery, and murder. This presentation, _Political Economy and Societal Consequences of Methamphetamine Epidemic in the United States,_ explores history, economy, and human costs associated with this drug. The presentation will then discuss the ways in which the drug has brought about manifold changes in areas that fall under the rubric of _political economy_ including legislation, medical care, child welfare, and substance abuse treatment, changes that have come about as a reaction to and result of methamphetamine_s significant impacts on society.

Designing Drugs for Neurological Disorders: TRH-based Neurotherapeutics

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Background: The trend in neurotherapeutic drug design is moving from a 'magic bullet' to a 'magic shotgun' approach, yet the naturally occurring neuroactive peptide thyrotropin-releasing hormone (TRH) has potential to act as a 'magic bullet' in the treatment of a wide variety of CNS disorders by virtue of its multifaceted homeostatic neurobiological actions. Clinical use of TRH is restricted, however, because of its short half-life due to degradation by TRH-degrading ectoenzyme (TRH-DE). We report the development of novel compounds that offer an attractive means to overcome this constraint.
Methods: The ability of novel synthetic peptides to act as TRH-DE substrates or inhibitors and/or central TRH receptor ligands was determined by kinetic analysis and radioligand binding assays. The *in vivo* effects of systemically injected peptides were examined in male Wistar rats (200–250 g).
Results: QSAR revealed replacement of His in TRH (Glp-His-ProNH_2) by Asn confers resistance to TRH-DE proteolysis and efficient TRH-DE inhibition. Addition of hydrophobic L-amino acids to the C-terminus of Glp-Asn-ProNH_2 led to TRH-DE inhibitors with K_i values in the nanomolar range. Replacement of these hydrophobic residues with their D-isomers yielded a set of first-in-class compounds that potently inhibited TRH-DE and also bound to central native TRH receptors with a high affinity. Systemic injection of the lead compound of this set (Glp-Asn-Pro-D-Tyr-D-TrpNH_2, 1mg/kg) antagonised barbiturate-induced narcosis; increased rat activity scores ($P<0.05$, vs vehicle controls, ANOVA with Dunnett's post test) and enhanced behavioural responses compared to TRH alone ($P<0.01$, Bonferroni's comparison post ANOVA) (n = 5–23).
Conclusion: The development of a dual action bullet that targets two key members of the TRH signalling system and mimics and enhances TRH actions opens up a possibility for realizing the neuropharmacological potential of TRH actions and contributes useful insights to rational drug design.

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Drug Absorption From The Small Intestine In Immediate Postoperative Patients

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Background: The effects of surgery on gastric emptying have been documented for a considerable time, but less is known about the effects in the small intestine. It is thought that there is minimal diminution in the absorptive capacity of the small intestine postoperatively, although there is no literature on drug absorption in the early period after surgery. This study investigated drug absorption from the small bowel in patients undergoing abdominal surgery.
Methods: A prospective study of patients undergoing major abdominal surgery in which patients acted as their own pre-operative controls was carried out. Patients were administered the test drugs, paracetamol and ^{99m}TcDTPA, pre-surgery and two days postoperatively. Small intestine transit times, plasma concentrations and other pharmacokinetic variables were compared using Student's paired t test. Two complementary studies were carried out to establish pharmacokinetic parameters.
Results: There were no significant differences in the pre- and postoperative values of t_{max}, AUC, and AUMC pre- and postoperatively, ($p > 0.05$). There were significant differences between the pre- and postoperative values of C_{max} ($C_{max\ (preop)} > C_{max\ (postop)}$; $p < 0.05$) and the pre- and postoperative values of MRT ($MRT_{(preop)} < MRT_{(postop)}$; $p < 0.01$).
Conclusions: Drug absorption from the small bowel in the postoperative patient does not differ significantly from its preoperative absorptive capacity.

The ACE-Inhibitor: True Magic Bullet From Myocardium To Endocardium

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The following outcome data with the use of angiotensin converting enzyme inhibitors will be presented:

1) Atherosclerosis: Primary and secondary prevention of myocardial infarction.
2) Peripheral vascular disease: Primary and secondary prevention data
3) Retinopathy: Data on the prevention of diabetic retinopathy with the use of ACE-inhibitors
4) Heart failure: Outcome data in ischaemic and non-ischaemic causes of heart failure
5) Rhythm disturbances: Data on the prevention and treatment of atrial and ventricular rhythm disturbances
6) Renal disease: Data on the prevention and treatment of renal disease

Prevention of type 2 diabetes mellitus

ODAM As A Diagnostic And Therapeutic Target For Human Breast Cancer

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Background: We have previously reported that the structurally novel *Odontogenic Ameloblast Associated Protein (ODAM)*, expressed in ameloblasts during late tooth developments and in odonotogenic tumors is also found in human breast cancer (Kestler *et. al.* 2008 Mol Med 14:318-326).
Methods: To investigate the possible role of ODAM as a biomarker of breast cancer, as well as a potential diagnostic and therapeutic target, we have tested the capabilities of our anti-ODAM mAbs to immunostain a) mouse mammary tissue from different stages of development; b) human breast cancer arrays, and c) biopsy specimens obtained from patients with stages I-IV breast cancer. Additionally, we have used dual micro-SPECT/CT to image mouse mammary tumor xenografts with an ^{125}I-labelled anti-ODAM mAb.
Results: Tissues from all stages of mouse mammary development except lactation expressed ODAM; further, a significant number of specimens contained in human breast carcinoma arrays also were stained by these reagents. Among 60 patient samples analyzed in a retrospective study, ODAM expression was significantly greater in advanced (stage IV) than in early (stages I-III) disease. The ^{125}I-anti-ODAM mAb was also capable of imaging a murine mammary xenograft. Furthermore, we found (using an ELISA-based procedure) that the sera of patients with metastatic breast cancer contained elevated titers of anti-ODAM antibodies.
Conclusions: Our finding that ODAM expression in human breast cancer correlates with disease stage has prognostic import**.** The presence of anti-ODAM antibodies in the sera of patients with metastatic disease also is of note, given that autoantibodies to growth regulatory factors have been detected in individuals with other types of malignancies in which the titers of these components have correlated with survival and other clinicopathological parameters. Additionally, radiolabeled anti-ODAM mAbs may prove useful to document the presence of tumor metastasis or relapse. Based on our data, we posit that ODAM has a functional role in breast development and in the pathogenesis of breast cancer where it could serve as a novel diagnostic and therapeutic target.

Treatment Prospects for Breast Cancer: Lessons Learnt from a Decade of Research on Maspin

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Background: Maspin is a multifaceted protein, interacting with a diverse group of intra- and extra-cellular proteins, regulating cell adhesion, motility, apoptosis, angiogenesis and critically involved in mammary gland development. Maspin is a cytosolic protein but is also localized to the nucleus and membrane, and is secreted. Aberrant methylation of the Maspin promoter is closely associated with *Maspin* gene silencing and is a common occurrence in cancer. Our laboratory has identified the aspartyl endopeptidase Cathepsin D (CatD) as a binding partner for Maspin. Unlike Maspin, which is downregulated in primary tumors and absent in metastatic breast cancer, CatD is excessively produced and aberrantly secreted by tumor cells. Studies depict a critical role for CatD in tumor growth (mitogenic effect), and invasion (proteolytic effect on matrix components) in breast cancer. Based on the importance of both Maspin and CatD, and specifically the reciprocity of their relationship in breast cancer, we embarked on identifying factors which might influence the Maspin and CatD partnership under normal conditions. Such an approach would shed light on how their alteration could lead to malignant growth and ultimately metastasis.
Methods: We employed *in vivo* (mice model of mammary gland development), and *in vitro* (normal mammary epithelial and breast cancer cell lines grown on 3D matrices) models to decipher the Maspin and CatD partnership in the context of mammary gland development and during neoplastic breast cancer progression.
Results: Our studies have illuminated a previously unidentified function for Maspin and its interaction with CatD in maintaining the differentiated secretory glandular phenotype of the mammary gland. In addition, the secretion of Maspin by mammary epithelial cells and its deposition into the extracelluar milieu plays an important role in matrix degradation by CatD. In this capacity Maspin could potentially regulate mammary tissue remodeling occurring under normal and pathological conditions.
Conclusions: Studying this unique partnership has provided us with a critical view into several previously unidentified mechanisms of action for both of these proteins, and may contribute new strategies underlying Maspin- and CatD-based therapeutic approaches for combating breast cancer.

Anti-inflammatory, analgesic and antipyretic activities of Physalis minima Linn

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Background: To evaluate anti-inflammatory, analgesic and antipyretic activities of Physalis minima Linn in order to discovery natural remedies for the management various painful and inflammatory conditions.
Methods: NMRI mice (22–28 g) and Wistar rats (180–200 g) of either sex were obtained from the animal house facility of H.E.J. Research Institute of Chemistry, University of Karachi, Karachi, Pakistan. The carrageenan induced hind paw edema and Cotton pellet-induced granuloma tests were conducted for both crude extract and chloroform fraction. While the Acetic acid-induced abdominal constriction and Formalin test were performed to evaluate the analgesic potential of crude extract and chloroform fraction. The crude extract and chloroform fraction was also tested against Brewer's yeast (Saccharomyces cerevisiae), induced fever.
Results: The crude extract (58%) and chloroform fraction (62%) of Physalis minima significantly inhibited the carrageenan induced pay edema in rats at 400 mg/kg. In a dose dependent manner at 400 mg/kg, the crude methanol extract and chloroform fraction reduced granuloma (48%) and (62%) respectively. In a dose dependent manner at 400 mg/kg, the crude extract and chloroform fraction reduced the number of abdominal constriction (52%) and (38%) respectively. The crude methanol extract demonstrated (51%) and chloroform fraction (31%) activity in dose dependent way in the late phase in formalin induced pain. In case of antipyretic assay, the crude extract and chloroform fraction of Physalis minima expressed insignificant activity. Values of $p < 0.05$ were considered significant in all cases.
Conclusions: Both the crude extract and chloroform fraction of the plant showed significant anti-nociceptiv and anti- inflammatory activity as compare to control, while the anti-pyretic response was insignificant.

Peptides Against Ageing

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Background: Ageing is characterized by desorganization of peptidergic system of regulation of organism functions. The development and study of peptide geroprotectors is very prospective.
Methods: The level of expression of various genes, intensity of protein synthesis in the cells, indices of immune and endocrine system, metabolism and antioxidant defense were studied in animals and humans.
Results: Peptides regulated gene expression and protein synthesis in the cells. This was largely conditioned by immunomodulating, oncomodifying and stress-protection properties of peptides. Peptides contribute to DNA structure restoration and decreased incidence of chromosome aberrations in the cells induced by radiation, chemical effects and hypokinesia. Mechanisms of their geroprotective action are related to activation of chromatin in blood lymphocytes of old patients. Peptides show opioid activity and produce modulating effect on the content of biogenic amines (noradrenalin, dopamine, 5-oxyindolacetic acid, serotonin, histamine) in brain cortex and blood serum of animals, due to their effect on the central and peripheral regulatory mechanisms of stress and inflammation. Geroprotective action of peptides is also related to their influence on the mechanisms of hormone regulation and antioxidant defense. Administration of thymus and pineal peptides to mice and rats of different strains promoted reliable increase in an average life span by 30-40% and depressed growth of spontaneous, induced and transplanted tumors in animals. Animals revealed restoration of melatonin level, antioxidant defense enzymes and normalization of some components of mitochondria respiratory chain. Administration of pineal peptides to old monkeys promoted reliable restoration of melatonin, cortisol and glucose in the blood to the level in young animals.
Application of thymus and pineal peptides in elderly and old patients resulted in restoration of melatonin level, indices of antioxidant defense, immune, endocrine and cardio-vascular systems, brain functions. It was accompanied by a 2-fold decrease in the mortality rate in these patients during 8-12 randomized clinical studies.
Conclusions: The results of studies evidence prospects for application of peptide geroprotectos for prevention of premature ageing, age-related pathology and an increase in the period of active longevity.

New Steroidal Hormones Promise to Become a Multi-Purpose "Magic Bullet"

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Background: Discovery of plant steroid hormones called brassinosteroids (BS) showed that steroids are versatile hormonal regulators, characteristic to most organisms inhabiting the Earth. BS demonstrate wide spectrum of regulatory effects on plant growth and development. Their important feature is the ability to enhance plant resistance to unfavorable factors (diseases, stresses, pollutants, etc.) and to improve plant productivity together with the quality of crops. BS protective action is a result of multiple changes at molecular and cell level including activation of protein and nucleic acids' biosynthesis, changes in hormonal balance, activity of enzymes, in composition and properties of membranes. As obligatory constituents of plants, BS have been and are consumed by mammals with food over all the evolution, but till recently there were practically no attempts to investigate their specific effects in higher animals except toxicological studies.
Methods: This paper reports our results in studies of effect of 24-epibrassinolide (EBI - one of the typical and most active BS) on the serum cholesterol levels, data on its anti-HIV activity *in vitro* and some other data reflecting its action on immune and hormonal system of animals. Effects on the serum cholesterol levels were studied in rats and human volunteers. Anti-HIV activity was studied using Formazan assay, Supravital cell staining by the trypan blue assay and Indirect immunofluorescence assay. Some effects of EBI on immune, hormonal and reproductive systems were investigated in mice, in rats and in chickens and in fishes.
Results: The study showed a high efficacy of EBI as a cholesterol-lowering agent in mammals for a wide range of doses. Tests on anti-HIV activity showed that EBI is efficient as anti-viral agent at average concentration of 10^{-7} mol/L. Effect on hormonal balance and stimulation of immune and reproductive system were registered.
Conclusions: The obtained data provide evidence that BS possess different activities in animals, and these activities are similar to a certain extent to those we know in plants both in respect to profile and active doses. This finding allows looking at BS from a new point of view: they are promising multi-purpose agents for human and veterinary medicine.

From 1-4 Weeks Of Treatment Down To A Single Application: A Novel Terbinafine Topical Treatment Of Tinea Pedis

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Background: Tinea pedis is a common dermatophytosis requiring topical antifungals for at least 1-4 weeks (W). This has a negative impact on compliance and outcomes. A novel topical solution of terbinafine (film-forming-solution - FFS) was developed to allow single application
Methods: 1- The stratum corneum (SC) pharmacokinetics (PK) of terbinafine following single application was investigated in three PK trials on healthy volunteers (n = 6, 12 and 18). Drugs were applied to the back and skin strips were taken from defined areas at baseline and from 1 to 312 h after application. Samples were analysed using validated liquid chromatography/mass spectrometry. **2-** Dose-finding and efficacy trials: 344 and 273 tinae pedis outpatients confirmed by mycological examination were evaluated for efficacy of 10% and 5% (dose-finding trial only) and 1% FFS in randomised double blind vehicle controlled parallel group trials. Evaluations were carried out at baseline, 1 and 6 W. Effective treatment rate (ETR) based on negative mycology and minimal symptoms was measured at W 6. In the efficacy trial, recurrence (positive cultures at W 12) was also assessed.
Results: 1- The residence time of the film on the skin was up to 72 h. 30% of the total amount of drug delivered into the SC occurred during the first 2 h, 31% from 2-12 h, and 39% thereafter. The maximum concentration was observed as early as 1.5 h. Fungicidal SC terbinafine levels were still detected after 13 days (24 ng/cm^2). **2-** ETR at W 6 with 10%, 5% and 1% FFS were 61%, 70%, 66% compared to 18% with the vehicle. All three active treatments were significantly superior to the vehicle (P<0.001). 1% and 5% FFS were non-inferior to 10% FFS. In the efficacy trial, ETR was 63% in the 1% FFS group and 17% for the vehicle (P≤0.0001). Recurrence occurred in 12.5% of the effectively treated patients at W 6. 1% FFS was well tolerated.
Conclusions: This novel formulation delivers high amounts of terbinafine to the SC for a prolonged time. 1% FFS was the minimal effective dose. Effectiveness of 1% FFS was confirmed by the efficacy trial which also showed a similar relapse/re-infection rate to that previously demonstrated with terbinafine 1 % cream for 1 week. This novel product represents a significant advance in the treatment of tinea pedis with the enhanced compliance and convenience that it offers.

Tumor Necrosis Factor (TNF)-α Inhibitors Effectively Treat Asthma

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The morbidity and mortality from asthma in the Western world have profoundly increased in the past two decades. Recent studies showed that sensitization and exposure to cockroach allergens strongly correlated with increased asthma morbidity and severity for children, especially among inner city children. As a unique form of chronic airways inflammation, asthma is characterized by reversible airway obstruction, airway hyperresponsiveness, and the production of multiple inflammatory mediators. Local activation of both immune and nonimmune cells in the lung triggers the release of these immunomodulator proteins including tumor necrosis factor (TNF) – α. TNF-α, as a multipotent pro-inflammatory cytokine, has been postulated to be a critical mediator directly contributing to the bronchopulmonary inflammation and airway hyper-reactivity in asthma. The successful treatment of various chronic inflammatory diseases such as rheumatoid arthritis, Crohn's disease, and psoriasis provides great potential that inhibition of TNF-α activity may have application for the treatment of asthma. Recently we have shown that airway expression of TNF-α peaked shortly after allergen challenge in a mouse model of asthma induced by a house dust extract that contains high level of cockroach allergen and endotoxin. TNF neutralization with a specific antibody significantly reduce the pulmonary inflammation and airway hyperresponsiveness. Recent developments in clinical trials in patients with severe asthma provide strong support for the concept that blocking TNF-α activity represents a new approach in asthma therapy.

Effect Of A Subtoxic Dose Of Acetaminophen On The Toxicity Of Chemicals That Are Metabolically Activated

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Background: While numerous studies describe the toxic consequences resulting from an excessively large dose of acetaminophen (APAP), a widely used analgesic-antipyretic, its potential adverse effects at a lower or therapeutic dose have hardly been explored. The present study was aimed to examine the effects of prior exposure to a subtoxic dose of APAP on the metabolic disposition and toxicity of a following dose of this drug.
Methods: In a preliminary experiment an APAP dose of 500 mg/kg, ip, was shown to be non-toxic to female Sprague Dawley rats used in this study. At 18 hr after administration of APAP at this dose, rats were challenged with an identical dose of APAP and the elimination of APAP from blood was determined. Serum enzyme activities were measured 24 hr after the challenging dose of APAP to estimate the liver injury. Also the hepatic microsomal drug metabolizing enzyme activities and their expression were measured in rats treated with a single dose of APAP 18 hr prior to sacrifice.
Results: APAP and APAP-glucuronide concentrations in plasma were unaltered by APAP pretreatment. APAP-sulfate concentrations were decreased, while APAP-cysteine concentrations were elevated significantly. The elevation of serum hepatotoxic parameters was also enhanced by APAP pretreatment. In rats treated with a single dose of APAP 18 hr prior to sacrifice, hepatic microsomal chlorozoxazone 6-hydroxylase, p-nitrophenol hydroxylase, p-nitroanisole O-demethylase, and aminopyrine N-demethylase activities were all increased to 173 %, 151 %, 158 %, and 116 % of normal control, respectively. Immunoblotting analysis indicated that expression of CYP2E1, 3A, and 1A was also induced significantly. Neither hepatic glutathione contents nor glutathione S-transferase activity was changed by the single dose of APAP.
Conclusions: 1) A subtoxic dose of APAP may increase the CYP2E1, 3A, and 1A expression and their metabolizing activities. 2) The altered CYP contents and activities may actually influence the metabolism and resulting toxicity of a repeated dose of APAP. 3) Considering the wide use of APAP as an analgesic-antipyretic, it is suggested that a greater concern should be expressed regarding the effects of acute or repeated dosing of this drug even at a therapeutic level, especially when used in combination with other medications.

Quinolone Resistance In *Campylobacter* Isolates Originating From Chicken In Senegal.

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Background: In Senegal, fluoroquinolones (norfloxacin and enrofloxacin) were first used in poultry production in 1996 to treat respiratory and intestinal diseases. It is known, however, that ciprofloxacin resistance in *Campylobacter* isolates derived from commercial chickens reached 40%. Aims: 1) To investigate the genetic basis of quinolone resistance in *Campylobacter* strains isolated from chicken in Senegal. 2) To determine the relationship between quinolone resistance and sequence type (ST).
Methods: This study included 54 *Campylobacter jejuni* and 33 *Campylobacter coli* isolates obtained from 14 dispersed collection sites over a 3-year period. The susceptibility of isolates to nalidixic acid and ciprofloxacin was determined by E-test and the agar dilution method. The quinolone resistance-determining regions (QRDR) of *gyrA* and *gyrB* genes were sequenced. Multilocus sequence typing (MLST) was used to study the clonality of isolates.
Results: Among the 27 ciprofloxacin-resistant *C. jejnui* isolates (MICs 8 to >32µg/ml), 18 exhibited the Thr-86-Ile substitution, 4 had the Thr-86-Ala substitution and 5 showed no mutation in the GyrA QRDR. However, two isolates susceptible to ciprofloxacin but intermediate to nalidixic acid (MIC 16 µg/ml) had also the Thr-86-Ala substitution in the GyrA protein. Two additional substitutions (Asn-203-Ser and Ala-206-Thr) were identified regardless of quinolone susceptibility. For *C. coli*, among the 14 ciprofloxacin-resistant isolates, 12 displayed the Thr-86-Ile sustitution, and 2 had no substitution within the GyrA QRDR. The sequencing of the *gyrB* QRDR from quinolone-resistant isolates revealed no substitution. MLST showed that the resistance phenotype varied for the same ST and within the same lineage.
Conclusions: 1) The Thr-86-Ile substitution in the GyrA protein was the predominant mechanism of quinolone resistance. However, some isolates displayed an unusual mechanism of resistance to quinolones. 2) There was no link between quinolone resistance and ST, and that the emergence of quinolone resistance is not related to the diffusion of a unique clone.

Opioid Agonist or Opioid Antagonist: Magic Bullets in the Treatment of Opioid Addiction

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Background: Methadone maintenance treatment for chronic opioid addiction was envisioned as a "magic bullet" to eliminate opioid withdrawal and craving to make meaningful rehabilitation possible. Methadone maintenance over long periods was undesirable to some patients, yet discontinuation of methadone maintenance often led to heroin relapse even after long periods of abstinence. So an alternate orally active "magic bullet," naltrexone, was developed to improve overall acceptability of opioid addiction treatment. It blocks the opioid receptor and prevents opioid physical dependence. Unfortunately, this highly efficacious treatment is poorly effective because patient adherence is worse than that for methadone maintenance. The effectiveness of both medications is significantly limited by patient non-adherence, so systems of care must be developed to ensure delivery of the medication and other services needed for optimal rehabilitation. This "magic gun" for the "magic bullet" is key to the treatment process, but is an element often lacking in treatment of chronic medical problems.
Methods: We developed Motivated Stepped Care (MSC) as a "magic gun" to improve the effectiveness of methadone maintenance by using the behaviorally reinforcing properties of methadone to motivate improved adherence to a stepped care, patient-treatment matching paradigm. Patients enter MSC in low intensity counseling care and are referred to higher, discrete intensities ("steps") based on objective indicators of current treatment response. Once stabilized, they are returned to lower steps of care in an efficient and cost effective manner. A randomized, controlled trial of 127 new admissions was used to evaluate the effectiveness of MSC.
Results: Patients randomly assigned to MSC (n = 65) had lower rates of poor treatment response (46% vs 79%, p < .001), and improved counseling attendance (83% vs 44%, p < .001) compared to a standard treatment condition (n = 62). MSC was well tolerated and associated with excellent attendance across varying treatment schedule intensities.
Conclusions: 1) Treatment adherence and response to MSC is superior to standard methadone maintenance treatment. 2) This treatment approach has broad theoretical and practical application in treatment of addiction and other chronic behavioral problems that share the common problem of poor treatment adherence.

Exploiting Plant Sources for Potential Drugs. Alpinumisoflavones in Perspective

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Background: Isoflavones/flavones have been known to have an array of medicinal properties and currently several medicines in allopathic medical practice are Isoflavones/flavones and their derivatives. Scientific investigations have established a myriad of biological activities justifying their concomitant use in folklore medicine several of which have been catalogued. In Franco West Africa, the bark infusion is used as a laxative for children, in Nigeria however, the pulverized root bark decoction is drunk as a relief for dysmenorrhea, as a de-wormer and blood purifier while the leaf extract is used as a cure for dysentery and diarrhea (Irvine, 1961; Abbiw, 1990). The leaf juice is reported to be lethal to Bulinus snail (Abbiw, 1990; Maillard *et al*., 1993), a water snail carrying the microorganisms that cause that cause schistosomiasis-bilharzias, a parasitic disease endemic throughout South America, Africa and the Far East. Our recent investigation into the structural and bioactive properties of selected alpinumisoflavones reveals a very strong brine shrimp lethality of these compounds (Kingsford-Adaboh et al., 2006). Brine shrimp lethality tests have been used for assaying potential anticancer candidates (McLaughlin JL *et al*., 1993). The discovery of the therapeutic potential of inhibitors of Monoamineoxidase-B in aging – related neurodegradative diseases such as the Parkinson's and Alzheimer's diseases has increased tremendously the interest in the search for compounds which have this therapeutic potential. It has been suggested elsewhere that the fruits of *Cudrania tricuspidata*, containing potent Monoamineoxidase-B (MAO-B) inhibitory prenylated Isoflavones, could be possible therapeutic candidates for the Parkinson's and Alzheimer's diseases. The chemical constituents of this plant which exhibited this high inhibition of MAO enzyme in concentration depended manner included 4-O-Methylalpinumisoflvone and Alpinumisoflavone which also occur in *Milletia thonningii* and other plants (Han XH et al., 2005). The potential antioxidant and anticancer properties of prenylated isoflavone have also been commented on (Comte *et al*., 2001).
In confirmation of the folkloric use of the West African Legume *Milletia thonningii* in Ghana and other parts of the world as anthelmintic and a purgative (Irvine, 1961; Abbiw, 1990), Perrett et al., (1995) found that the chloroform extract of the seed of *Milletia thonningii* which contain predominantly alpinumisoflavones elicited molluscicidal and cercaricidal activity when topically applied to mouse skin 2 and 24h prior to exposure to Schistosoma mansoni cercariae. The presence of the *Milletia thonningii* extract components on the skin appeared to be effective in preventing subsequent establishment of infection. This anti-schistosomal activity bioactivity has been corroborated (Maillard *et al*., 1995 and Lyddiard et al., 2002). Lyddiard and Whitfield mentioned and inhibitory effect of the crude extracts on site I mitochondrial electron transport system (Lyddiard and Whitfield, 2001). While these bioactivities have been unambiguously established, one is still at sea as to which of the many isoflavones are responsible for these bioactivities and how the presence, absence or relative positions of functional groups as well as differences in the molecular conformation are linked to these bioactivities. As part of our multidisciplinary approach towards the study and systematic characterization of the crystals of this plant, seven compounds have been studied some of which were isolated from the dichloromethane extracts of the rootbark and the seeds (Kingsford-Adaboh et al., 2001, 2006; Harrison et al., 2008) with the hope that the crystal structure, molecular and electronic properties can deepen our understanding of their observed bioactivities. The scheme below shows the compounds so far studied.

Methods:

1. Sample preparation and crystallization

Seeds of M. thonningii collected from the University of Ghana Botanical Gardens were air dried. These were ground into powder which was continuously soxhlet-extracted in methanol. Column chromatography and preparative thin layer chromatography yielded compounds (II) and (III), O,Odimethylalpinumisoflavone and 5-O-methyl-4-O-(3-methylbut-2-en-1-yl)alpinumisoflavone, respectively. Compound (I),4-O-methylalpinumisoflavone, which hitherto had been difficult to crystallize for X-ray diffraction purposes, was obtained from demethylating a product of (II) in a mixture of cold BCl_3 and chloroform. IR and NMR spectra confirmed the methylated product. These were crystallized from ethanol. For compounds (IV)- (VII) which were obtained from the demethylation, products obtained was divided into four and each recrystallized using acetonitrile, methanol, ethanol and water and the melting points of the compounds determined. The solid-solid phase transition temperatures between the solvent included compounds and the efflorescent crystals were measured by differential scanning calorimetry apparatus.

2. X-ray diffraction experiments

X-ray data of the compounds(IV,V,VI and VII) were collected on a *Rigaku R-AXIS RAPIDII* imaging plate diffractometer with graphite monochromated Mo Kα radiation (*λ* = 0.71075 Å). The absorption corrections were carried out using multi-scan (ABSCOR; Higashi, 1995). Cell refinement was carried out using PROCESS-AUTO and the data reduced by using Crystal Structure (Rigaku/MSC, 2004). The structures were however solved using SHELXS97 (Sheldrick, 2008) and the structures refined with SHELXL97 by full-matrix least-squares on F^2 against ALL reflections.

3. Toxicity measurement of samples (I),(II) and III)

Toxicity of the isolated compounds to brine shrimp Brine shrimp, Artemia salina Leach, has been successfully used as a bench-top assay to determine the toxicity levels of natural products from plants (Meyer et al., 1982), as brine shrimp larvae are sensitive to small doses of biologically active
chemicals. Eggs of brine shrimp (Brine Shrimp Direct, California, USA) were hatched in sea salt water (3.8%) and were allowed to hatch and mature for 48 h, before nauplii were used for a bioassay,in which dilutions of the compounds ranging between 0.01 and 100 mg ml^{-1} were used.

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Pharmacogenetic diagnostics for optimization of psychotropic drug treatment

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For many drugs, pharmacogenetic polymorphisms are known affecting biotransformation and clinical outcome. The clinical importance of these variants depends on allele-frequency and the effect size of the clinical outcome parameters. Further, it depends on the therapeutic range of the drug which is affected, on predictability of drug response as well as on duration until onset of therapeutic efficacy. Consequences which arise from genotyping might be: adjustment of dose according to genotype, choice of therapeutic strategy or even choice of drug.
For many psychotropic drugs, pharmacogenetic polymorphisms are known affecting biotransformation and clinical outcome. In antidepressant drug treatment, most drugs are metabolized via the polymorphic cytochrome P450 enzymes CYP2D6 and CYP2C19. Huge differences in pharmacokinetic parameters have been consistently shown for many tricyclics, some SSRIs, and other antidepressant drugs. However, the effects on therapeutic efficacy and adverse events have been described controversially. Pharmacokinetic differences caused by genetic polymorphisms can be overcome by adapting the drug dosages and dosing intervals. Similar to bioequivalence studies, the aim to achieve similar plasma concentration time courses of antidepressants might help to reduce side effects and therapeutic failure.
In the field of antipsychotic drug treatment, genetic polymorphisms in drug metabolizing enzymes as well influence pharmacokinetic parameters to a large part. In these kinds of drug therapy, a more clear dose dependency of side effects such as extrapyramidal side effects exists, and the consideration of genetic polymorphisms might be more beneficial. Recent studies showed a relationship between the occurrence of adverse antipsychotic drug effects and *CYP2D6* genotype. A prospective evaluation of the cost-benefit of genotyping in this field would be very helpful for the aim of introducing pharmacogenetic diagnostic into drug therapy.

Anti-Carbohydrate Specific Immune Response And Tumor Cell Lysis Correlate With Vaccination-Induced Systemic Release Of Stimulatory Cytokines

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Background: Tumor-associated antigens resulting from aberrant glycosylation, such as the SialylTn antigen, are frequently expressed on cancer cells and provide potential targets for vaccination. Carbohydrates, however, being T-cell independent antigens, are poorly immunogenic and fail to induce a memory response. To increase immunogenicity, SialylTn antigen was coupled to a highly immunogenic carrier, the murine monoclonal antibody mAb17-1A. An immunogenic formulation of SialylTn-mAb17-1A conjugate on alhydrogel (designated MB 402) with or w/o additional adjuvants was tested in Rhesus monkeys for tolerability and immunogenicity.
Methods: The SialylTn antigen was coupled to the mAb17-1A (mIgG2a) carrier. The coupling product was analyzed by SEC-HPLC, LDS-PAGE, Western blot, and IEF analysis. SialylTn-mAb17-1A conjugate was adsorbed onto aluminum hydroxide and co-formulated with QS-21.
Safety, tolerability and immunogenicity of multiple injections of MB 402 were evaluated in Rhesus monkeys vaccinated four times by s.c. injection and re-boosted on day 226. Blood samples were taken before and after immunization for serum analytic.
Immune response against mAb17-1A, SialylTn and Ovine submaxillary mucin, and SialylTn(+) tumor cells were measured. Cytokine release in serum was analyzed using xMAP Multiplex technology. NK lysis of tumor cells was measured using a ^{51}Cr-release assay.
Results: Immunization induced a strong immune response against the carrier but only IgM immune response against the SialylTn carbohydrate antigen. Co-formulation with QS-21 adjuvant, however, dramatically enhanced the anti-SialylTn immune response and resulted in a SialylTn-specific IgG switch. Cell binding and ADCC of SialylTn positive tumor cells was induced. The kinetics of carbohydrate-specific IgG response correlated with a temporary release of cytokines in the serum. NK mediated cytotoxicity against tumor cells was found.
Conclusions: The present study demonstrates that synthetic vaccines eliciting specific immune response against defined target antigen(s) together with a synchronized cytokine release are promising candidates for cancer vaccines.

Organ Independent Drug Elimination

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Background: Cisatracurium is a bisisoquinolinium nondepolarizing neuromuscular blocking agent used to facilitate tracheal intubation. Cisatracurium has been used for skeletal muscle relaxation during surgical procedures and/or during mechanical ventilator support. It has been shown that the major route of cisatracurium clearance is through the organ independent process of Hofmann elimination (pH and temperature dependent). Hofmann elimination takes place ubiquitously in plasma and tissue, and accounts for approximately 77% of the overall clearance of cisatracurium.[1] Tight physiologic control of pH and temperature, maintaining Hoffmann elimination, results in low interpatient variability in the CL of cisatracurium (16 %).[2] The aim of this study was to examine the relationships between pharmacokinetic parameters in the setting of organ independent elimination.
Methods: Geometric regression analysis was performed to look at the relationships between clearance and half-life, volume of distribution and half-life, and clearance and volume of distribution in 31 otherwise healthy patients who underwent minor surgical procedures requiring tracheal intubation.
Results: For cisatracurium, geometric regression analysis revealed that there is no relationship between clearance and half-life (n = 31; r^2 = 0.01) nor is there a relationship between cisatracurium volume of distribution and half-life (n = 31; r^2 = 0.01). However, cisatracurium clearance and volume of distribution were related (n = 31; r^2 = 0.54; p < 0.001).
Conclusions: Cisatracurium being "removed" from the body mainly by the organ independent route of Hoffman elimination results in unique relationships among pharmacokinetic parameters. Conventional relationships between pharmacokinetic parameters, with the primary parameters of CL and Vd, being independent and the secondary parameter of t1/2 being dependent on CL and Vd, do not hold for cisatracurium as the half-life is essentially "fixed" by Hoffman elimination. Half-life is independent of the CL and Vd, while the CL and Vd are directly related. Other compounds that undergo organ independent elimination via a ubiquitous mechanism may also display these unique characteristics with respect to pharmacokinetic parameter relationships.

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The Effect Of Some Endogenous Substances (Cl^-, Oleate And Ca^{2+}) On The Albumin Binding Of Triflupromazine (TFZ), Trifluoperazine (TFPZ) And Bendroflumethiazide (BFZ). An In Vitro Spectrometric Study

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Background: Albumin, the most abundant protein in the blood, binds several endogenous and exogenous substances reversibly, and thus is a carrier of these substances in the body. It is well known that most of administered drugs are bound to albumin, and that the only unbound free drugs can move into the tissues. Long-chain fatty acids (LCFA), such as oleate, and inorganic ions exist abundantly in the blood, and as they bind to albumin, it must be studied whether their binding may affect the binding of drugs to change the unbound free drug concentration.
Methods: Human serum albumin (HSA, fatty acid free), and fluorine-containing these drugs studied were purchased from Sigma. Second-derivative (DS) ultraviolet spectra of the drugs in HSA buffer solutions (pH 7.40) were measured to calculate the binding constants (Ks). Structural study of the drug binding and the effects of the endogenous substances on it were performed by ^{19}F NMR spectrometry.
Results: A single sharp ^{19}F NMR signal of each drugs in buffer solutions was split and broadened by addition of albumin, revealing that the drugs bind at more than one site. From competitive ^{19}F NMR experiments using known ligands, TFZ and TFPZ were found to bind to site I and another unknown site, and BFZ was to bind to both site I and II. Some of the ^{19}F NMR signals of the bound drugs showed intensity reduction or increase upon addition of the endogenous substances. The K-values (TFZ, TFPZ) measured in the presence of Cl^- (physiological concentration of 0.1 M) reduced to about 65% of the values measured without Cl^-. Addition of oleate increased the K-values depending on the amount of oleate up to three times of that of albumin, however, further addition reduced them. Ca^{2+} induced concentration- dependent suppression effect on the drug binding (TFZ, BFZ).
Conclusions: 1) Buffers to be used in the determination of the drug-albumin biding constants should contain 0.1 M Cl^-, otherwise, the binding constants may be over estimated. 2) The results of oleate suggested that the unbound free drug concentration may very depending on the amount of LCFA in the blood, which depends on physical conditions of the body. 3) Possible concentration fluctuation of Ca^{2+} in the blood may affect the free drug concentration.

KIT-Deficient Mouse And KIT-Targeted Drug

KITAMURA Y

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Mast cells were found by Paul Ehrlich and interstitial cells of Cajal (ICCs) by Ramon y Cajal, who also received the Novel Prize in 1906. We found deficiency of mast cells in W/W-v mutant mice, and thereafter the W locus was identified to encode the KIT receptor tyrosine kinase. Unexpectedly, W/W-v mutant mice lacked ICCs as well, indicating that the KIT signaling was essential for development of both mast cells and ICCs. Since we found gain-of-function mutations of the KIT gene in mouse and human mast cell tumors, we attempted to find tumors derived from ICCs. We identified the gastrointestinal stromal tumor (GIST) as such a tumor, and at the same time, gain-of-function mutations of the KIT gene in human GISTs. Unexpectedly again, a KIT-targeted drug, imatinib, was already present and has been successfully used for the treatment of GIST patients.

Genetic And Epigenetic Effects Of Pharmacological Doses Of Gamma-Hydroxybutyrate (GHB) In The Rat Brain

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Background: Gamma-hydroxybutyrate (GHB) is at the same time an endogenous neuromodulator of some brain synapses at micromolar concentrations, a therapeutic substance used to induce deep sleep in narcoleptic patients or for anesthetic purposes, an agent that has been proposed to alleviate withdrawal symptoms in alcoholic, but also a substance of abuse which can induce addiction. In order to obtain therapeutic or recreative effects, large doses of GHB must be administered or absorbed. The principal targets of these pharmacological doses of GHB are primarily the brain endogenous GHB system and the GABAergic system. However, it can be predicted that millimolar brain concentrations of GHB will targets multiple proteins and finally adapts the expression of several genes.
Methods: The present study focuses on the transcriptome modifications and HDAC inhibition due to GHB overload in two brain regions, the hippocampus and frontal cortex of the rat, after acute administration of pharmacological doses of GHB. These modifications were explored by microarrays analysis and concern 248 genes which showed 1.5 or greater changes in expression in the two brain regions.
Results: If we exclude the large proportion of modified EST (54.9% of all probes set), numerous functional important genes were differentially regulated: These concern first neuronal signaling and metabolic processing, then regulation of DNA transcription, stress response and neuronal growth or structure. Several differences exist between hippocampus and frontal cortex, but some genes are also similarly affected in both structures. As it become evident that important epigenetic mechanisms are at the basis of modifications in gene expression and regulate drug addiction, we demonstrate that acute pharmacological doses of GHB increase histone H3 acetylation in both brain structures. These results were confirmed by quantitative immunocytochemical studies and by in vitro HDAC inhibition by GHB.
Conclusions: The present study completes the classical model which described pharmacological effects of GHB and shows that acute doses of this substance exerts a profound effect at the level of genes transcription, probably partly due to modifications of the accessibility of chromatin to regulatory factors.

Si Quantum Dots functionalized for siRNA delivery in Caco-2 cells

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Background: Quantum dots are becoming popular as replacement for fluorescent dyes in biological fluorescence imaging because of their superior stability against photobleaching. The potential biocompatibility of silicon makes photoluminescent silicon quantum dots an ideal candidate for biological fluorescence imaging and versatile biomedical applications to human tissue. Quantum confinement of excitons in silicon nanoparticles smaller than 4.3 nm allows for tuning the luminescence wavelength in the visible range due to suitably adjusting the silicon quantum dot (Si qdot) size. One of the central challenges in silicon-based nanomedicine is to develop water-soluble Si qdots tailored for gene delivery, as image contrast agents and for diagnostic purposes.
Methods: For applications as image contrast agents and as transfection reagents for RNA interference (RNAi) the Si qdot surfaces were terminated with covalently linked ethenyl pyridine providing water solubility and positive charging. The surface chemistry and luminescence properties of the terminated Si qdots were characterized using FTIR and luminescence spectroscopy, respectively. The ability of the Si qdots to form complexes with siRNA was examined by gel electrophoresis. The Si qdot uptake by Caco-2 cells were investigated using TEM and confocal luminescence scanning microscopy. Downregulation of expression levels of MDR1 mRNA was determined by PCR.
Results: Ehthenyl-pyridine terminated Si qdots with mean sizes of 2.5 nm were observed to exhibit luminescence peaking at 520 nm. The uptake of ethenyl-pyridine terminated Si qdots by Caco-2 cells were shown by imaging cell slices and living cells with TEM and confocal luminescence scanning microscopy, respectively. The terminated, positively charged Si qdots were shown to bind negatively charged siRNA through their base function and were observed to enter Caco-2 cells via endocytosis. The siRNA molecules were released in the cytoplasm, where they participated in the RNAi pathway by targeting mRNA and therewith suppressing MDR1 gene expression. Complementary evidence for surpressed MDR1 gene expression were obtained from monitoring the reduced pharmacodynamic reponse of P-gycoprotein to rhodamine 123 with time-resolved fluorescence spectroscopy.

Cutaneous Microdialysis As A Useful Tool To Evaluate Skin Penetration Of Antiinfective Agents

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Background: Treatment with antiinfective agents will be successful if the concentration of active drug and/or its metabolite in the target tissue will be above the minimum inhibitory concentration and below the toxic one. From the dermatological point of view concentrations of the drug in skin seems to be of importance. Aim of our study was to evaluate skin concentrations of two important drugs: antiviral – acyclovir as well as antibacterial and antiprotozoal – metronidazole following a single oral dose of the parent drug assessed by cutaneous microdialysis. Moreover, the penetration of each unchanged drug into skin was compared with its penetration into theoretically calculated peripheral compartment.
Methods: To evaluate skin concentrations after a single oral dose of 0.4 g acyclovir or 2.0 g metronidazole linear microdialysis probes with 2 kDa molecular-weight cut-off were inserted intradermally to 20 healthy drug-free volunteers divided into two groups. The probes were perfused with Ringer solution. Concentrations of the drugs were determined by HPLC with spectrophotometric detection
Results The average maximum concentrations of acyclovir in the plasma and skin were 3.16 and 0.94 μmol/L, respectively, and were found after about 1.6 and 2.4 h. The pharmacokinetic parameters differed significantly between skin and theoretical peripheral compartment.
The average maximum concentrations of metronidazole in the plasma and skin were 214 and 151 μmol/L, respectively, and were observed after about 2.1 and 2.8 h. In contrary to acyclovir, pharmacokinetic parameters of metronidazole did not differ significantly between skin and theoretical peripheral compartment.
Conclusions: In certain cases concentration of the drug in the skin should be determined instead of its plasma concentration. However, evaluation of skin concentrations cannot be replaced by their concentration in theoretical peripheral compartment.

Novel Generation of Antivirotics: Combination of Antimetabolic and Immunostimulatory Modes of Action

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Background: Acyclic nucleoside phoshonates (ANPs) are recognized class of novel compounds used in therapy of viral infections. The major mechanism of antiviral action is the inhibition of virus-induced DNA polymerases or of reverse transcriptases. The prodrugs of compounds *tenofovir* and *adefovir* were approved for treatment of AIDS and hepatitis B, respectively. The aim of the present study was to investigate possible immunobiological activity of ANPs, i.e. up-regulation of nitric oxide (NO) production and cytokine secretion and to compare the effects in cells of animal and human origin.
Methods: ANPs were synthesized in-house (Inst. Organic Chemistry and Biochemistry). Pooled peritoneal cells (PECs) were collected from female mice of the inbred strain C57BL/6 (4-8 mice/treatment). The sources of human peripheral blood mononuclear cells (PBMCs) were buffy coats from healthy donors. The animal PECs were cultured at final density of 2.0 x 10^6/ml, the human PBMCs at density of 1.0 x 10^6/ml in complete RPMI-1640 culture medium. The cells were cultured in presence of varying concentrations of ANPs (5-50 μM). Levels of cytokines in supernatants of mouse and human cells were determined by ELISA. The length of culture was usually 16 h. The concentration of nitrites in supernatants of mouse cells was taken as a measure of NO production detected after the 24-h culture (a Griess reagent).
Results: Several ANPs have been found to stimulate secretion of TNF-α, IL-10, RANTES and MIP-1α with good correlation between mouse PECs and human PBMCs: r = 0.969 (P < 0.0001) and r = 0.982 (P < 0.0001) for MIP-1 and RANTES. ANPs significantly augment production of NO primarily triggered in mouse peritoneal cells by IFN-γ. The effect is closely associated with their ability to stimulate secretion of cytokines. Highly significant correlation exists not only between the range of NO production and extent of cytokine stimulation in animal cells but also between NO in mouse and cytokines in human cells: r = 0.958 (P < 0.0001) and r = 0.969 (P < 0.0001) for MIP-1 and RANTES, respectively.
Conclusions: Acyclic nucleoside phosphonates are potent imunostimulators and thus represent new generation of antivirotics with a dual, i.e. antimetabolic and immunostimulatory modes of action.

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Discovery Of Dual-Targetting Ligands

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Background: A wealth of examples, both in literature and in the clinic currently exist whereby drugs have been shown to act via multiple targets, albeit these discoveries were generally retrospective. The intentional design of ligands that act at dual or multiple targets is becoming widely recognised as an approach to produce a single drug, in some cases with superior side-effect profiles and therapeutic effects, when compared with the combination of individual drugs. In general, methods such as generation of a conjugate ligand that incorporates functional groups key for binding to both targets are employed, with efficacy. However, this method often leads to the identification of higher MW compounds, as pharmacophores for both targets are not significantly overlapped.
We present a computational approach consisting of 'Target Fishing' concepts combined with Virtual Screening to converge on dual-targeting molecules with highly integrated pharmacophores for different unrelated protein families: Estrogen Receptor alpha/Tubulin and finally Hsp90/Tubulin.
Methods: Firstly, 'Target-Fishing' methods were employed to identify molecules with similar characteristics to known active ligands for the Estrogen Receptor for example, from a database containing a large number of molecules with known activities for certain biological targets (WOMBAT). Having identified the targets to which those molecules belong, we set about developing and optimizing Virtual Screening platforms combining cheminformatic, docking and pharmacophoric softwares to screen for molecules that 'hit' both targets.
Results: The first screen allowed identification of a molecule that binds potently (1.4nM) to the Estrogen Receptor and also to Tubulin. Importantly, we found that this compound is actually a naturally occurring compound contained in citrus fruits. A second screen to identify molecules that modulate both Hsp90 and Tubulin was undertaken. A micromolar binding compound to both targets is revealed along with preliminary biological data on cell lines. Application of these methods should allow identification of more novel molecules targeting other dual or multiple proteins/enzymes in the future.

The Pharmacology, Pharmacokinetics, Clinical Efficacy, Adverse Effects And Toxicities, Drug Interactions, Dosage And Administration, And Safety Issues Related To The Use Of Prasterone Are Discussed

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Prasterone is a proprietary synthetic dehydroepiandrosterone product under investigation for use in women with systemic lupus erythematosus (SLE) who are taking glucocorticoids. Initial trials investigated prasterone as a treatment to improve disease activity and symptoms in women with mild to moderate SLE. The Food and Drug Administration (FDA) did not approve prasterone's labeling for these indications. Subsequent trials have focused on prasterone as a treatment to limit bone loss in women who have SLE. A study was conducted to assess bone mineral density in patients who had been taking glucocorticoids for six months or longer. The patients in the prasterone group showed an increase in bone mineral density, while the placebo group demonstrated a loss. The most common adverse effects of prasterone therapy were acne and hirsutism. Hematuria, hypertension, and serum creatinine concentration increases have also occurred. Interactions of prasterone potentially exist with 5-alpha reductase inhibitors and additive or antagonistic effects could possibly occur with androgens, estrogens, oral contraceptives, and progestins. In clinical trials, oral prasterone dosages of 100-200mg /day were administered. These dosages have resulted in supraphysiological hormone levels.
Conclusion:
FDA has granted orphan drug status for the prevention of loss of bone mineral density in SLE patients taking glucocorticoids. FDA is requesting additional Phase III trial data for the treatment of SLE and the prevention of loss of bone mineral density.
Source:
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Targeting Cell Cycle Progression by Troglitazone

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Background and Aim: Increasing evidence has confirmed that ligands for peroxisome proliferator-activated receptor γ (PPARγ) exhibit antitumoral effects through inhibition of cell proliferation and induction of cell differentiation in several malignant neoplasms. Recently, we have documented the accumulation of a cyclin-dependent kinase inhibitor, p27*Kip1*, as well as an unexpected accumulation in cyclin E in G1-arrested human hepatoma cells treated with the PPARγ ligand troglitazone. Simultaneous accumulations in both p27*Kip1* and cyclin E are known to be characteristic phenotypes in cells derived from mice lacking Skp2, an F-box protein component of the SCF ubiquitin-ligase complex. Thus, the aim of the present study was to assess whether Skp2 might be involved in the up-regulation of p27*Kip1* in troglitazone-treated human hepatoma cells.
Methods: Human hepatoma cell lines were used in this study. Cell cycle was analyzed by flow cytometry and immunoblotting. The mRNA levels of p21, p27, and cyclin E in the hepatoma cells were examined by quantitative real-time RT-PCR. Skp2-overexpressing hepatoma cells were generated by the cDNA transfection. The expression levels of Skp2 and p27*Kip1* in human hepatocellular carcinoma (HCC) tissues and the adjacent noncancerous liver tissues were assessed by immunoblotting.
Results: A striking decrease in Skp2 expression and a reciprocal increase in p27*Kip1* expression were found in troglitazone-treated hepatoma cells but not in those cells treated with other PPARγ ligands such as pioglitazone and ciglitazone. Quantitative real-time RT-PCR analysis showed that troglitazone downregulated Skp2 at the mRNA levels. Consistently, ectopic overexpression in Skp2 brought resistance to troglitazone, resulting in a decreased population of arrested cells at the G1 phase compared with that in the mock-transfected cells. In surgically resected hepatocellular carcinoma (HCC) tissue, an increased expression in Skp2 was found in both the moderately differentiated HCCs and the poorly differentiated HCCs.
Conclusions: Troglitazone attenuated Skp2 expression, thereby promoting p27*Kip1* accumulation in human hepatoma cells. This therapeutic potential of the ligand may lead to new cell-cycle-based antitumor strategies for advanced HCCs.

Enhanced Potency Of Antibodies Using Biologically Active Peptides

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Background: Antibodies have evolved as effective alternative to chemical drugs to treat cancers, yet clinical response is limited by expression of tumor targets reducing the potency of antibodies. We have invented methods to enhance potency by (1) generating polyvalent (homophilic) antibodies and by (2) making membrane penetraing antbodies, The objectives of these methods is to create antibodies with enhanced anti-tumor effects and for intra-cellular targetsto control cell proliferation.
Methods: To demonstrate enhanced potency of homophilic antibodies the Her2/neu specific Herceptin was crosslinked with a homophilic 26mer peptide derived from a naturally ocuuring homophlic antibody, T15, previously discovered in our laboratory. The peptide was photo-actively affinity crosslinked to Herceptin. Homophilic Herceptin was compared to naked Herceptin in FACS, apoptosis induction and in xenograft animal model using the human lung tumor H1650. Transmembrane antibodies were generated by photo-affinity crosslinking a 14mer peptide derived from human sarkosi sarcoma virus. Intracelluar targeting of life cells is demonstrated by confocal microsopy and inhibition of induced apoptosis.
Results: Homophilic Herceptin poduced > 1 log stronger fluorescent intensity over naked Herceptin and increased the number of apoptotic cells to 82% compared to 12% with naked Herceptin. Homophilic Herceptin inhibited tumor growth by 60% in H1659 xenograft experiments compared to mice treated with naked Herceptin or in no-treatment controls.
Trans-membrane penetrating (TMP) antibodies stained in live cells specifically actin and paxillin while naked antibodies did not. TMP antibodies also did not affect cell growth in culture. Furthermore, TMP modified anti-caspase3 antibodies blocked induction of apoptosis induced by actinomycin.
Conclusions: 1) Photo-affinity crosslinking of biologically active peptides endows antibodies with properties that enhance targting and opens up novel target choices. Homophilic peptide modified Herceptin is more efficient in tumor killing in vitro and in vivo than naked Herceptin. 2) Membrane penetraing modified antibodies target intra-cellular antigens in living cells. 3) Collectively, these data provide methods to create a novel class of antibodies that are superior as diagnostic tools and therapeutic drugs.

Antioxidant and Antiglycation Potential of Some Sudanese Medicinal Plants and their Isolated Compounds

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Background: Free radicals or reactive oxygen species (ROS) appears to be associated with a number of human neurodegenerative disorders, inflammation, diabetes, viral infections, autoimmune pathologies and digestive system disorders. In the present some of Sudanese medicinal plants commonly used in the folk medicines against infectious diseases were investigated for their potential antioxidant, antiglycation and cytotoxicity.
Methods: In the present work twenty three parts from 20 medicinal plants were extracted by 80% ethanol. These extracts were were investigated for their potential scavenging of superoxide free radicals by using PMS–NADH (phenazine methosulphate -nicotinamide adenine dinucleotide hydrogen reduced form) systems by oxidation of NADH and assayed by the reduction of NBT (nitroblue tetrazolium). The inhibition of glycated proteins was carried for the above extracts after long term icubation of glucose with BSA (bovine serum albumin). MTT {3 - (4, 5-dimethylthiazole – 2 – yl) – 2, 5 – diphenyl tetrazolium bromide} cytotoxicity assay was conducted for the above extracts against 3T3 cell line. Some of the acive extracts were subjected to phytochemical analysis for the isolation of active princibles by using column chromatographic techniques.
Results: 14 extracts were revealed significant activity P < 0.5 for the scavenging of superoxide free radicals. *A. nilotica* barks, *B. aegyptiaca* barks, *K. senegalensis* leaves were the most potent with 74.7, 72.1, 70.5 % inhibition respectively. However, the rest revealed moderate inhibition activity. Only *A. nilotica* barks (78%) , *K. senegalensis* barks (74%), *A. nilotica* fruits (66%) and *T. bakis*. (61%) were reveled over 50% inhibition of glycation production assay, while the rest were less effective. No any significant reduction observed for tetrazolium of MTT cytotoxic test from such extracts. Sixteen compounds of different groups were isolated from acive plants and screened for the above bioassays.
Conclusions: 1) *A. nilotica* barks extract was found to be the most potent antioxidant and antiglycation among all examined extracts 2) Bioassay guided phytochemical investigation proved catechin was the most potent isolated compound for both scavenging of superoxide free radicals and inhibition of glycation production assay3) MTT cytotoxicity against 3T3 cell line indicates the safety of all plant ethanolic extracts as well as isolated compounds.

IgY (Immunglobuline from egg-Yolk) - a "magic bullet" to fight antibiotic resistance

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> *Paul Ehrlich, the father of sera therapies, searched for substances that could seek up specific disease-causing agents and link with toxins like a key in the lock. He found antibodies circulating in the body like "magic bullets"*

The European Antimicrobial Resistance Surveillance system states, that the largest threat to health in Europe today is antibiotic-resistant bacteria. Each year 3 million Europeans are affected by infections in medical care, and approximately 50,000 of them die. The cause of this terrible development is excessive use of antibiotics and a continuous use of antibiotics will accelerate the rate of resistance. It is therefore extremely important to encourage a more restricted use of antibiotics (www.ecdc.europa.eu/documents/pdf/01_2006_AR_web.pdf). The increasing number of antibiotic-resistant bacteria emphasises the need to find alternative methods. Immune therapy can be used either alone or as a complementary treatment to antibiotics.
Specific IgY has attracted considerable attention as an alternative to treat infectious diseases. Its biochemical properties make it attractive for passive immunotherapy. Hens vaccinated with bacteria or other antigens produce specific IgY against them. A single hen produces ~ 40 g IgY per year in their egg yolk. IgY preparations are stable for years. IgY antibodies decrease bacterial adhesion to epithelial cells and neutralise toxins **Efficacy:** Specific IgYs have proven efficacy against several enteric bacteria, viruses and fungi in animal and human studies. Specific IgY is effective also against antibiotic resistant microbes. In our own studies specific IgYs have been effective against Pseudomonas aeruginosa, Candida albicans and Enterobacter faecalis. **Safety:** Orally given immunoglobulines are not absorbed from the gastrointestinal tract. Orally administered IgY neither activates the human complement system nor reacts with any cell activators or mediators of inflammation. IgY is generally recognised as safe for oral or local use. There have not been any adverse events in our anti-pseudomonas study over 12 years to patients with Cystic fibrosis (CF). Individuals, who are allergic to eggs, must be excluded. **Diminishes the use of antibiotics:** In our hands IgY reduces the use of antibiotics and thereby also the severe drawbacks of antibiotics: Development of resistant strains, disturbance of the microbiologic flora, toxicity and allergenicity. There is no risk that pathogens develop resistance against IgY. **State of the art:** The Swedish Medical Agency has granted licence for anti-pseudomonas IgY to a group of CF patients. EMEA has approved orphan drug designation for Anti-pseudomonas IgY to treat patients with CF. ***Conclusion:*** IgY offers great opportunities to treat specific infectious diseases and thereby diminish the need of treatment with antibiotics. IgY is, indeed, one of Paul Ehrlichs "magic bullets".

Nitric Oxide and Zidovudine Potentiate Oxidative Response of Stimulated Macrophages

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Background: Macrophage is one of the key cell types involved in many disreases. Therefore modulation of oxidative response (respiratory burst) of stimulated macrophages by various pharmacological agents is very important. The aim of this study was to test (separately) modulation of respiratory burst by zidovudine and nitric oxide *in vitro*.
Methods: Macrophages were isolated from the peritoneal cavity of 300-400 g male Sprague Dawley rats 3 days following injection of casein. The cells were washed and incubated with 18 μM of nitric oxide donor S-nitroso N-acetylpenicillamine (SNAP) for 3 hours, or azidothymidine (AZT) and azidothymidine monophosphate (AZT-MP) from 10 μM to 1 mM for 18 hours. The oxidation of 2',7'-dichlorodihydrofluorescein (DCDHF; 5 μM) to 2',7'-dichlorofluorescein by activated cells was measured with a fluorescent plate reader using a 24-well tissue culture plate. Cells were activated by adding 1 or 10 μl of a 10% suspension of polystyrene latex beads per million cells. Determination of hydrogen peroxide produced by non-activated and activated macrophages was based on horseradish peroxidase-mediated oxidation of phenol red.
Results: Latex-activated cells oxidize DCDHF due to release of hydrogen peroxide and low-molecular iron complexes, which was verified in experiments using inhibitors catalase (1000 U/ml), desferal (100 μM) and peroxidase inhibitor sodium azide (2 mM). Preincubation of macrophages with SNAP (18 μM, 3 hours) increased DCDHF oxidation by latex-activated cells compared to control untreated cells. This effect was absent in macrophages incubated with SNAP decomposition products for 3 hours. SNAP also increased hydrogen peroxide release by activated cells. We have observed significant and dose-dependent increases in DCDHF oxidation in cells incubated with AZT, or AZT-MP. Catalase (100 U/ml) addition during incubation of cells with latex beads removed the effect of AZT and AZT-MP on DCDHF oxidation compared to control cells. Furthermore, AZT and AZT-MP increased hydrogen peroxide release from activated cells.
Conclusions: Separate application of nitric oxide donor and zidovudine enhances oxidative potential of activated macrophages.

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Serine Protease Inhibitors: Cross Inhibitors of Prokaryotic and Eukaryotic Systems

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Background:β-Lactams have historically been viewed as a class of antimicrobials. However, this paradigm is shifting towards a focus on their ability to function as inhibitors of bacterial enzymes, particularly those involved in broad-spectrum β-lactam resistance, i.e., extended spectrum β-lactamases (ESBL). This shift in focus is the result of recognition that β-lactam's acylate a broad range of enzymes that extends beyond taxonomic kingdom boundaries. These compounds have demonstrated activity against viral and mammalian serine enzymes in addition to their tradition target of bacterial enzymes. The common theme for these □-lactam targets is that the majority of the enzymes have serine as nucleophile in the active site. The focus of this presentation is on the evaluation of the potential of β-lactam antibiotics as inhibitors of the serine enzymes of both prokaryotic and eukaryotic origin, with specific focus on the structure-function relationship of β-lactams as antimicrobial and antineoplastic agents.
Methods: Molecular modeling studies and stereoselective synthesis have aided the design and synthesis of monocyclic β-lactam libraries as inhibitors of prokaryotic and eukaryotic enzymes, which allowed for determination of the structure activity relationships.
Results: The ability of the β-lactams to act as excellent acylating agents is centered on the four-membered β-lactam ring, while the substituents at the C3 and C4 positions provide certain specificity for recognition by the corresponding enzymes.

Conclusions: A rigid confirmation is a common thread for β-lactams functioning as enzyme inhibitors. Conformational requirements for recognition by proteases suggest a fundamental platform for the preparation of inhibitors that is dependent on developing conformationally restricted inhibitors which adopt receptor-binding conformation, and are therefore entropically advantaged for binding to a protease. However given this basic requirement for activity, selectivity has been difficult to achieve due to the similarity in the active sites. This double-edged sword will make the future development of inhibitors at once both easier and more difficult because of the potential for inflicting the very damage use of these inhibitors are hoping to block.

The Pharmaceutical Policy In Context Of Health Care System In Albania

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The purpose of this study is to provide an in depth analysis on the current situation of the Pharmaceutical Sector , to identify the strengths and weaknesses in this sector and also to formulate a proposal document with the main strategic guidelines of Pharmaceutical Policy for present and future reference.
My research is focused on some main problems of the pharmaceutical policy. In particular it covers pharmaceutical market, general prescribing, public private roles at the pharmaceutical sector , the approval procedure of drugs and legislation, pharmaceutical expenditure and reimbursement of drugs, price and distribution, management drug in hospital, health insurance and access to drugs , national pharmaceutical policy etc.
The changes in Albanian Politics during 1990-1991, created a new atmosphere in all economic and social sectors, because Albania had inherited a list of unresolved problems and emerging complexities.
Such problems are related to:

- Outcome (equity, efficiency, satisfaction problems)
- Output (quality and amount of services)
- Input structure(resource problems)

On 1995, The Health Insurance Institute was established, and functioning as a "single payer" scheme. For the first time the general practitioners and essential pharmaceuticals were insured under the Health Insurance Institute.
During the transition period in our country a lot of problems were faced in the pharmaceutical market, but we would like to highlight most important such as the strengths and weakness as follow:
Strengths:

- The privatization of the pharmaceutical sector – flexibility, diversity, improvements in services and meeting patients needs.
- Structure of the pharmaceutical sector in a country level;
- Pharmaceutical distribution covers the whole country;
- Privatization of the pharmaceutical industry.
- Compilation of the pharmaceutical legislation;
- Formulation of the Pharmaceutical Order;
- All the medicine for sale have the price tag attached to the label;

Weaknesses:

- A document of pharmaceutical politics with all its necessary components does not yet exists.
- Pharmaceutical sector functions like a regular market (demand- supply of pills) and not like a medical service with the health as the main concentration.
- The market is dominated by the symptomatic medicines instead of the etiologic ones relevant to the diseases.
- Almost 60% of the total Health Insurance Fund goes to the pharmaceutical sector to cover the reimbursed drugs.
- The production capacity of the drugs in our country is very low, and the production quality does not meet the criteria of GMP;
- There are no any written and approved standards by the Ministry of Health for the Pharmaceutical sector.
- The environment where the medicines are stored, like drug stores, warehouses and other pharmaceutical agencies do not always meet the health regulations.
- Lack of communication and misunderstanding between doctor-pharmacist-consumer.
- Uneven distribution of the drug stores in the country, most of them located in Tirana;
- There is missmanagement of the medicines in the hospitals;
- An education and training program of the staff does not exist in the institutional level;
- There is a lack of management and financing of the Research & Development filed.

More changes are necessary in the pharmaceutical sector to insure the collaboration among all institutions, as well as the government support in improving all the necessary legislation related to the pharmaceutical sector. The pharmaceutical Policy should play an important role in Health Care System in Albania.

Experimental Approach for Growth Inhibition of Human Malignancies by the Highly Efficient Anti-tumor Peptides Delivery System

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Background: Molecular targeting agents have become formidable anticancer weapons, which show much promise against the refractory tumors. Functional peptides are among the more desirable of these nanobio-tools. Intracellular delivery of various functional peptides forms a basis for potent, non-invasive mode of delivery, providing distinctive therapeutic advantages.
Methods: We examined growth suppression efficiency of aggressive human leukemia/lymphomas, glioblastomas and other human malignancies in vitro and in vivo tumor models by introducing anti-tumor peptides as a complex with the „Wr-T" peptide transporter which serves to augment delivery of a cargo peptide. We did single, or dual peptide introduction of two tumor suppressor peptides ($p14^{ARF}$, $p16^{INK4a}$, $p21^{CIP1}$ functional peptide or their combinations) using Wr-T-mediated intracellular peptide delivery.
Results: Wr-T–mediated transport of $p16^{INK4a}$ functional peptide dramatically inhibits growth of highly aggressive p16-negative leukemia/lymphoma cell lines by up to 80% through restoration of p16 function. Based on this result, we further did Wr-T-mediated simultaneous introduction of two tumor suppressor peptides, $p14^{ARF}$ and $p16^{INK4a}$ functional peptides, into human glioblastoma cell lines, which reversed specific loss of *p14* and *p16* function, thereby drastically inhibiting tumor growth by >95% within the first 72 h, whereas the growth inhibition was ~40% by p14 or p16 single-peptide introduction. Additionally, the combination of p16 and $p21^{CIP1}$ peptides dramatically suppressed the growth of glioblastoma line which carries a missense mutation in *p53*, by >97% after 120 h. Significantly, our murine brain tumor model for dual-peptide delivery showed a substantial average survival enhancement ($P < 0.0001$) for peptide-treated mice. The similar inhibitory effect by dual peptide introduction was also observed in p16- and p14-double negative cancer cells.
Conclusions: Thus, it was demonstrated that the peptide transporter-mediated delivery of single or dual anti-tumor peptides seems to be highly effective against aggressive human malignancies in non-invasive manner, by singly or jointly restoring multiple tumor suppressor functions.

pH Dependent 5-Fluorouracil Release System using Polymeric Micelles

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Background: The newly formed tumor vessels are usually abnormal in form and architecture, and tumor tissues usually lack effective lymphatic drainage. Therefore, these factors will lead to abnormal molecular and fluid transport dynamics especially for macromolecular drugs and nano carrier. On the other hand, it has been known that amphiphilic block copolymers consisting of a hydrophilic and a hydrophobic block can form core-shell micelles, referred to as "Polymeric Micelles", in a selective solvent due to the association of the insoluble block. Polymeric micelles have attracted much attention in drug delivery systems. However, only a few works on controlled drug release from polymeric micelles has been studied. Aims: 1) To fabricate the polymeric micelle decomposed in an acidic condition. 2) To investigate the 5-fluorouracil (5FU) release profiles from the polymeric micelles with the change of pH.
Methods: The amphiphilic block copolymer was synthesized by the mechano-chemical solid-state polymerization of poly 4-vinylpyridine and methacryloyl galactopyranose. Polymeric micelles were prepared by the dialysis method. Dynamic light scattering measurements were performed to observe the size change of polymeric micelles with pH. The 5FU released from polymeric micelle was monitored with UV spectrometer.
Results: The number average particle diameter of polymeric micelles prepared was about 200 nm. The particle diameter of polymeric micelles steeply decreased at pH 5.6 (less than 10 nm), although its diameter unchanged from pH 7 to 5.7. When the pH was changed from pH 1 to 7, the particle diameter steeply increased at pH 5.6. It was also shown that 5FU was immediately released from the polymeric micelle at pH 5.6, although 5FU was not detected from pH 7 to 5.7 within a detectable extent.
Conclusions: 1) The polymeric micelle prepared from this amphiphilic block copolymer was immeadiately deforemed or formed at pH 5.6. 2) 5-Fluorouracil incorporated in polymeric micelles was steeply released at pH 5.6. Therefore, this polymeric micelle seemes to be applicable for the pH dependent drug release system. 3) It is well-known that lysosome is acidic environment. Thus, this polymeric micelle is expected as lysosome targetting system.

Comparative Study of Dihydroartemisinin and Artesunate Safety in Healthy Thai Volunteers

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Background: Artemisinin derivatives including artesunate and dihydroartemisinin (DHA) have been used for treatment of malaria. As part of new drug development initiatives in Thailand, a new tablet formulation of DHA has been developed. Our initial phase I bioequivalence study indicated that the new and reference DHA formulations were well tolerated; however, a significance decrease in hemoglobin (0.7-0.9 g/dl) was detected after a single 200 mg oral dose. To explore further, a clinical study with an emphasis on hematological parameters was conducted.
Methods: A single-center, randomized, single-blind, cross-over clinical study was conducted in 24 healthy Thai volunteers with a dosage of 300 mg daily for 2 days. Artesunate was used as a comparator. Clinical adverse events were monitored. Laboratory assessment (CBC, RBC morphology, reticulocyte count, Coombs'test, total and direct bilirubin, LDH, AST, ALT, and hemoglobinuria) was performed on study days 0 prior to drug administration and days 2, 5, and 7 post drug administration.
Results: Eighteen volunteers (10 males and 8 females) completed both rounds of the study. All adverse events were mild. Nausea and vomiting were common and occurred only in female volunteers. A statistically significant decrease in hemoglobin was detected (0.5 g/dl at study day 7, $P < 0.05$). Decreases in laboratory values below the normal limits were observed in some volunteers for reticulocyte and WBC counts. Other changes in laboratory values were minor. Transient, mild bone marrow suppression was evidenced by: 1) reduction of reticulocytes, 2) leukopenia, and 3) a minor drop of platelet counts.
CONCLUSIONS: The present study confirmed our previous finding on a significant decrease in hemoglobin. Bone marrow suppression might be one of the causes. Considering the absence of clinically significant anemia (though decrease in hemoglobin) and its similarity in drug response profiles to artesunate, the development of the DHA should pursue.

Energetics of Cytochrome P450 Hydroxylations: Making Sense of *In Silico*:

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Cytochrome P450 catalyzes, among others, the reaction RH + O_2 +H^+ + NADPH $\rightarrow$ ROH + H_2O + $NADP^+$, in which RH may be a drug. As most drugs interact with cytochrome P450, there are three possibilities: (*a*) inactivation of cytochrome P450, (*b*) inactivation of the drug by cytochrome P450, and (*c*) activation of the drug by cytochrome P450. An ideal drug, or magic bullet, is to be activated, because an effective drug that is inactivated by cytochrome P450 requires an additional drug, namely an inhibitor of cytochrome P450. Use of a pro-drug may indeed be a wise strategy as cytochrome P450 is overexpressed in some cancer cells. The thermodynamic properties of the intermediates in the cytochrome P450 cycle can be estimated with a few simple assumptions, and the result for the electrode potential of the couple Compound I/Compound II is 1.4 V (W. H. Koppenol, *J. Am. Chem. Soc.* **129**, 9686-9690; 2007), a value in agreement with an estimated bond enthalpy $D(FeO–H)^{3+}$ of ca. 410 kJ/mol (M. T. Green, J. H. Dawson, and H. B. Gray, *Science* **304** (5677), 1653-1656; 2004). Compound I is almost isoenergetic with the haemiron(III) – hydrogen peroxide complex that precedes it. The electrode potential of 1.4 V is quite different from that implied by the results of *ab initio* calculations. A higher electrode potential would imply a small association constant between the haem iron(III) and hydrogen peroxide, and a lower value would not allow hydroxylation. Compound I can thus abstract a hydrogen of a primary carbon atom; abstraction from a secondary or tertiary carbon atom is thermodynamically more facile. *Thus, hydroxylation to activate a drug is thermodynamically always feasible, and is only limited by the regiospecificity of the hydroxylation.* If hydroxylation is undesirable, because it inactivates the drug, see (*b*) above, then, rather than adding a compound that inactivates cytochrome P450, one might attempt to replace the hydrogen that is abstracted with a fluor atom.

Ab Initio Calculation Of Molecular States Of Compounds Of The Lanthanum And Yttrium Molecules

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The potential energy curves of the grounds and lowest electronic states of the compounds of the Lanthanum and Yttrium Molecules have been investigated via CASSCF method. Multireference CI calculations (single and double excitation with Davidson corrections) were performed. Lanthanum and Yttrium atoms are treated in all electron scheme. In the range of internuclear distance R around the equilibrium distance of their ground states, the molecules are assumed to be mainly ionic. The potential energy curves for the considered states in the representation $^{2s+1}\Lambda^{(\pm)}$ have been calculated in the range 2.0Å $\leq r \leq$ 3.5Å. The spectroscopic constants such as the vibrational harmonic constant ω_e, the internuclear distance at equilibrium r_e, the rotation constant B_e, and the electronic transition energy with respect to the ground state T_e have been calculated by fitting the energy values around the equilibrium position to a polynomial in terms of the internuclear distance, the degrees of these polynomials are determined from the evaluation of the statistical error for the coefficients. By using the canonical functions approach and the cubic spline interpolation between each two consecutive points of the potential energy curves obtained from the *ab initio* calculation, the eigenvalue E_v, the rotational constant B_v, the centrifugal distortion constants D_v, and the abscissas of the turning point (R_{min}, R_{max}) have been calculated for various vibrational levels. The comparison of these values to the theoretical and experimental results available in the literature shows a good agreement. Many electronic states for the considered molecules have been studied theoretically for the first time.

Electronic Homeopathic Preparations (EHPs) as Potential "Magic Bullet": Pilot Study on Biologic Model

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Background: Medicinal and diagnostic preparations based on electronic-homoeopathic copying from parent substances have received a certain distribution in complementary medicine in spite of significant deficit of objective scientific data on their biomedical effect. EHPs specific action without side effects is declared. The aim is to test the effect of EHP with biological model of tomato seedlings.
Methods: For double blinded randomized trials were handled to estimate influence of water-based EHP of organic fertilizer Biohumus and Placebo (P) on growth and weight of tomato seedlings. The electronic-homeopathic copying was carried out with "Simulator" (Metabolics Ltd, GB) apparatus by the same operator. The tomato seeds were soaked in preparations then planted out in plastic boxes by 40-49 pieces. Equal exposition of plants to external conditions was achieved. The groups of plants were fed by preparations (200-300 ml) once a week by the same researcher. On the 38-th day of each trial the plants were cut up and the length of green part of each plant was measured with a ruler. The mass of each plant was determined by electronic weighing. Nonparametric Mann-Whitney U-test and Wald-Wolfovitz runs test (*) were used to analyze differences between EHP and P.
Results: Statistical significance of differences in trials is shown in the table.

Trial No.	No. 1	No. 2	No. 3	No. 4
Mass	n/s	p = 0.00016	p = 0.014*	p < 0.0001
Growth	n/s	p < 0.0001	n/s	p = 0.028

Direction of EHP action re P differs from trial to trial. So, in trial No.2 and No.3 the EHP action intensifies the development of plants re P, and conversely in the trial No.4 it weakens the development of plants re P. It is supposed that electronic-homoeopathic copying phenomenon may be related with operator's body being a source of wide-band electromagnetic disturbances associated with the vital activity of cells and organs which are modulated by parent substance during copying.
Conclusions: 1) Revealed in 3 independent double blind randomized trials from 4 significant differences in the results produced by EHP and P points to the reality of electronic-homoeopathic copying phenomenon. 2) The multiplicity of effects produced by EHP re P has been in different trials.

The distribution of antimicrobial resistance patterns of nasopharyngeal *Haemophilus influenzae* isolated from healthy preschool children

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The ecosystem of nasopharyngeal microflora is the reservoir for bacterial pathogens, *e.g. Haemophilus influenzae*, involved in community-acquired respiratory tract infections, especially in young children. These infections are most frequently endogenous in origin. No literature data are available for resistance rates among nasopharyngeal *H. influenzae* isolated from healthy young children. In addition, day care centre attendance has been reported as a major risk factor for increased rates of carriage of some bacterial pathogens, including drug resistant strains and for increased prevalence of respiratory tract infections.
The aim of the present work was to study an antimicrobial resistance of *H. influenzae*, isolated from throat or nose of 343 healthy preschool children (3-5 years old): 266 children from four day care centers (D group, including subgroups: D1 - 85, D2 - 62, D3 - 44, D4 - 75) and 77 children staying at home (control group, C). For identification of the bacterial isolates routine methods were used. Susceptibility of the tested bacteria to antimicrobial agents was determined by the disc diffusion procedure. Production of beta-lactamases was detected with nitrocefin test.
The prevalence of *H. influenzae* positive children was 18.18% in C group and 21.05% in D group. 71 isolates of *H. influenzae* were isolated: 14 from C group and 57 from D group (D1 – 9, D2 - 15, D3 - 7, D4 – 26). 31 (43.66%) of the isolates were resistant to one or more antimicrobials: 7 (50%) from C group and 24 (42.11%) from D group: D1 - 6 (66.67%), D2 - 5 (33.33%), D3 - 2 (28.57%) or D4 - 11 (42.31%). The high rate of resistance to trimethoprim/sulfamethoxazole was found - 21.4% in C group and 29.8% in D group. 8 (11.27%) of the isolates were ampicillin-resistant (C group – 3, D group – 5), including 7 - beta-lactamase positive (C group - 3, D group – 4), and 1 from D group – beta-lactamase negative, resistant to ampicillin and amoxicillin/clavulanic acid. Some of the isolates demonstrated resistance to the other beta-lactams (e.g. II or III generation of cephalosporines or monobactams).
The drug resistance pattern of nasopharyngeal *H. influenzae* isolated from healthy young children may be useful in prediction of drug resistance pattern of *H. influenzae* of clinical specimens and in consequence, for selection of proper antimicrobial agents used for empiric treatment of respiratory infections caused by haemophili rods. Besides, these data confirm that drug-resistant strains, being a part of normal microflora, can be considered as a reservoir of resistance genes.

Enveloped Virus Neutralizing Compounds (EVNCs), The Magic Bullets against a Broad Spectrum of Deadly Viruses Causing a Billion Infections Annually Around the globe

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Background: Enveloped viruses like the human Immunodeficiency virus (HIV), influenza viruses including H5N1, hepatis viruses, HBV and HCV, and Herpes viruses 1 and 2,I together cause at least over a billion infections annually in humans across the globe. Most current antivirals and vaccines are specific for a certain type of virus and are susceptible to resistance due to hypermutation. In addition, a number of antiviral agents have serious and debilitating side effects that need to be carefully managed under costly state-of-the-art medical facilities and supervision that are normally not widely available in the developing world. With this in mind, my collaborators from around the world and myself, set out to screen possible natural antiviral compounds that will be a) broad spectrum, b) not susceptible to resistance, c) have minimal or no short term and long side effects and d) not require costly management. We screened several compounds including lectins and mucins and have focused our attention in characterizing the structure, efficacy, safety, stability and mechanism of action of EVNCs from fulvic acid (FA) and pomegranate juice (PJ).
Methods: Our general method of testing is to treat one million virus particles with 0.5%-2% of the FA or PJ for 1-5 min at room temperature or 37 degree centigrade and to estimate viral infectivity with and without treatment. In order to determine whether the EVNCs can effectively neutralize a broad spectrum of enveloped viruses, we have tested antiviral activity of EVNCs against an attenuated vaccinia virus vGK5, cowpox virus, influenza including H5N1, SARS virus. HIV, herpes viruses 1 and 2 and are currently testing against hepatitis C virus. In order to test the short term safety we have used cell toxicity assays as well as *in vivo* studies and to test longterm safety we have used the Ames test for mutagenesis. In order to test heat stability we have tested the efficacy before and after autoclaving the FA and PJ solutions and to study the mechanism of action we have done kinetic studies. In order to isolate the bioactive compounds we have significantly enriched the compounds by passing through HLB copolymer columns with hydrophilic-lipohilic balance (Waters Oasis) and have analyzed the fractions for antiviral activity and structural analysis by NMR. **Results:** The EVNCs show antiviral activity against vGK5, SARS virus, HIV, influenza and herpes viruses at the concentration tested 0.5% to 2%. The antiviral activity is stable at the temperature of autoclaving which is 121 degree centigrade. The EVNCs can neutralize genetically diverse strains of influenza pr8, X31, H5N1 at concentrations of 0.625%, suggesting that it will be resistant to hypermutation which are charactertic of influenza. The short term toxicity studies showed that at concentrations at which the EVNCs are active there is no cell toxicity and survival and health profile of mice was not affected by a 100 fold therapeutic dose. Long-term toxicity studies showed that while the unfiltered FA had mutagenic activity, the ultrafiltered FA with 3 kDa cutoff Amicon filters did not have any long-term toxicity. **Conclusions**: The EVNCs have a broad spectrum antiviral activity against enveloped viruses and genetically diverse influenza viruses tested. The bioactive agent is possibly acidic (in the case of PJ, probably ellagic acid) has been enriched and its structure is being elucidated. Short erm and long term studies suggest that the EVNCs are safe and non toxic. The EVNCs has potential in the development of preventive and prophylactic vaccines against certain enveloped viruses as well as as microbicides against sexually transmitted diseases and as orally administered therapeutics.
Authors' disclosure statement:
The author has a potential interest in the global production and distribution of the EVNCs and has applied for protecting the intellectual property arising from this work. The author is the President of the companies (InflaMed Inc., and kbiotech) that would be doing Research and development of the EVNCs for therapy, vaccines and microbicides, based on this work. The EVNCs and their source, FA and PJ are experimental agents at this time and therefore no claims are or can be made about actual human use and therefore not for human use.

Bacteriologic and therapeutic aspects of paediatrics osteo-articular infections.

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Background: osteo-articular infections provided several injuries and interest all the joints bone and the paediatric skeletal. Successful osteo-articular infection treatments need effective antibiotherapy which depends of the causal bacteria and their poor treatment can lead to important functional sequela.
Objective: Report the causal bacteria of the ostea-articular infections treated in our department
Method: Retrospective study of 1486 osteo-articular infections treated during 18 years has been performed at the general paediatric surgery of Yopougon, Abidjan Cote d'Ivoire. The age average was 7 years ± 4, sex-ratio was 1,2. The osteo-arthritis was observed in 77% and osteomyelitis in 23%. The sickle cell disease was detected in 9% and HIV infection in 0, 01%. The first line antibiotic for medical treatment associated oxacillin to thirst generation of cephalosporin. Surgery treatment was indicated when pus collections were documented radiologically. Biologic examinations consist of the blood culture, pus culture, C reactive protein, VS and blood count cell. We studied the osteo-articular infection response to the first line antibiotic according bacteriologic characteristic of the causal bacteria (type of bacteria, the bacterial resistance and the antibiogramm). Bacteria identification was performed according classic procedure and antibiogramm was performed with Orisis Biorad with CASFM references (committee of antibiogramm of French microbiology society).
Results: The blood culture was positive in 31% and the pus culture was positive in 13%. The causal bacteria were, ***Staphylococcus aureus*** in 23%, ***Salmonella*** in 17 %, ***streptococcus pneumo*****nia** in 13%, ***Citrobacter freundii*** in 9%, others bacterias in 20%. The first line antibiotherapy was effective in 25% and antibiotic resistance was observed in 33%. The bacteria which produced betalactase with a large spectrum was observed in 15%, classic quinolone resistance was observed in 15% and the cross resistance to the quinolone in 50%. ***Staphylococcus aureus*** was methicillino-resistant in 25%, and negativ gram bacteria resitance in 40%.
Conclusion: The first antibotherapy line with oxacillin and thirst generation of cephalosporin indicated osteo-articular infection is less effective because of the bacteria resistance.

Anti-microbial, Anti-diarrhoeal and Toxicity Profile of Cylicodiscus gabunensis Stem Bark (Mimosaceae)

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Background: Diarrhea is a major health problem for children worldwide, accounting for 5-8 million deaths each year. *Cylicodiscus gabunensis* (CG) is a plant of Cameroonian pharmacopeia. It is reputed for its beneficial effects in the treatment of diarrhoea related illnesses. But their use as medicament based simply on a traditional folk use that as been perpetuated along several generations with no scientific data on their efficacy. Since sub-acute or sub-chronic toxicity data are required to predict the safety associated to the use of medical products, it was necessary to provide this information in order to bridge the gap in knowledge about the toxicity profile of this plant. Therefore the present study has been planned to investigate the real efficacy and safety of this drug. **Methods:** In order to be sure of the therapeutic properties that this plant may have as anti-diarrhoeal, the ethyl acetate (EA) extract of the stem bark of (CG) was evaluated *in vitro* for its anti-microbial activities against 17 pathogenic microbial strains involved in diarrhoeal infection isolated from patient : *Escherichia coli, Klebsiella pneumoniae, Shigella dysenteriae, Shigella flexneri, Morganella morganii, Proteus vulgaris, Proteus mirabilis , Salmonella typhi, Citrobacter freundii , Enterobacter cloacae, Enterobacter agglomerans , Staphylococcus aureus , Streptococcus feacalis , Pseudomonas aeruginosa, Bacillus cereus T, Candida albicans* and *Candida glabrata*. Disc diffusion method was employed for the determination of anti-microbial activities and MICs (minimum inhibition concentration) against the test organisms by broth macrodilution method. The effect of the (EA) extract of (CG) was further investigated for anti-diarrheoal activity *in vivo* against castor oil-induced diarrhoea, charcoal-induced gut transit and castor oil-induced small intestine enteropooling. The electrolyte concentration of the small intestinal fluid was also evaluated. The toxicity profile of the (EA) extract of the stem bark of (CG) was studied on wistar rats. The rats were administered graded doses (0.75, 1.5, 3 and 6 g/kg p.o) of the extract daily for 6 weeks and the effects on clinical signs, body weight, food and water consumption, organ weight, haematology, histology as well as serum, hepatic and renal biochemical parameters were measured. **Results:** The best MIC and MBC values for the microorganisms sensitive to the extract were 0.00078 and 0.00315 mg/ml respectively. The greater and remarkable antimicrobial activity of the (EA) extract of CG was recorded with *Staphylococcus aureus, Proteus vulgaris* and *Bacillus cereus T.* Like loperamide (3 mg/kg body weight) a single oral dose of this extract (375, 750 mg/kg body weight) produced a significant decrease in the severity of diarrhoea. The extract produced a decrease in intestinal transit (10.26 - 30.75 %,), and unlike atropine, it significant inhibited castor oil-induced enteropooling. However, it did not alter the electrolyte concentration in intestinal fluid as compared to castor oil-treated rats. Body weight of dosed and control rats increase throughout the duration of treatment but food and water consumption were not significantly affected. The relative weights of the liver, lungs, heart and kidneys remained normal whereas a significant change was observed in that of the spleen. The hematocrit level was increased in treated animal. Our data demonstrates a significant increase in serum concentrations of aspartate amino-transferase, alanine amino-transferase, total cholesterol and glucose with high-dose of CG treatment tested (3 g/kg). CG also caused a significant reduction in hepatic malondialdehyde concentration. Renal urea and creatinine levels were reduced significantly in test groups. Histological findings reveal a characteristic progression treatment-related effect on liver, kidneys and lungs. The acute toxicity LD_{50} was estimated at 14.5 and 11 g/kg body weight for male and female respectively, but dose-related mortality of 30 and 50 % was observed during the sub-acute toxicity. **Conclusion:** 1- Our observations confirm that (EA) extract of the stem bark of (CG) possesses strong anti-microbial activity *in vitro* against some pathogenic microbial strains involved in diarrhoeal infection.2- It also possesses a significant anti-diarrhoea activity due to its inhibitory effect both on gastrointestinal propulsion and fluid secretion. 3 - The findings on the toxicity study have once more highlighted the limitations of the acute toxicity LD_{50} testing and suggest that (CG) may exert varied toxicological effects when administered orally either acutely (up to 4 g/kg p.o.) or sub-chronically (up to 0.75 g/kg p.o.) in rats.

Secretin and Autism

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Background: Leading morphological disorders in autism are found in the cerebellum and in the frontal and parietal cortices. In autistic postmortem cerebellum the level of AMPA glutamate receptors, GAD and reelin level decreased compared to control values. Increased homovanillic acid level in the cerebrospinal fluid, hyperserotoninemia and endorphinemia, changed serotonin metabolism in the brain are the most consistent disorders in autism. The researchers look for compounds to treat autism. At this moment there are no therapeutic agents which are able to generally improve the autistic phenomena. Inspite of this fact there are many compounds that are used for treating autistic patients and at least in part of the cases they seem to be effective. One of these compounds is secretin. Its significance in autism was first suggested by Horvath and his co-workers (1998, 1999). Their observation facilitated us to explore the occurrence of secretin in the nervous system.
Methods: In our laboratory with the use of immunohistochemistry secretin immunorectivity was looked for in colchicine treated rats, intact cat and human samples. We have also investigated the role of secretin given intracerebroventricularly *(icv)* on the behaviour of mice with genetic cerebellar atrophy testing open field activity, novel subject approaches and rearing.
Results: Secretin was found in the pyramidal cells of the motor cortex, Purkinje cells, a subpopulation of central cerebellar nuclei, the mesencephalic nucleus of the trigeminal nerve, the superior olivary nucleus, the cells of trapezoid body, a subpopulation of the cells of spinal and trigeminal ganglia. Our data show, that besides a well established synthesis of secretin in the gastrointestinal tract, several nerve structures can produce secretin. *Icv* administration of secretin ameliorated the hypermotility of mice which is characteristic for this genetically modified strain.
Conclusion: Our data and those available in the literature indicate that secretin, besides its gastrointestinal role, is a neuropeptide as well. It was found by Gershon and D'Antreaux (2003) that the number of secretin producing S cells in autistic patients is half of the normal. Because secretin is present in Purkinje cells, its level has to be dramatically decreased in the case of cerebellar atrophy.
Tthe low level of secretin may be responsible for some autistic symptomes.

Vitamin A and D Derivatives; Potential MAGIC BULLETS with Antithrombotic and Antineoplastic Applications

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Background: Tissue factor (TF) is a membrane-bound glycoprotein that is essential for activation of the coagulation pathway. Although synthesis of TF is tightly regulated, its expression can be induced by a variety of agonists, such as tumor necrosis factor (TNF) and oxidized low-density protein (LDL). Induction of TF expression in endothelial cells, monocytes, or malignant cells is associated with fatal thrombotic disorders, such as disseminated intravascular coagulation (DIC) and atherosclerotic thrombosis. Downregulation of the pathological expression of TF appears to be a critical strategy to prevent and treat various thrombotic disorders. A vitamin A derivative, all-*trans* retinoic acid (ATRA) and the active form of vitamin D_3, 1□,25-dihydroxyvitamin D_3 (D_3), and their synthetic analogs can induce myeloid leukemia cells to differentiate and inhibit the clonal growth of those cells.
Methods & Results: Because of dramatic clinical experiences of rapid amelioration of DIC in acute promyelocytic leukemia patients treated with ATRA, we found the anticoagulant effects of RA derivatives. They evoke an anticoagulant effect by upregulating the expression of an anticoagulant glycoprotein thrombomodulin (TM) and downregulating the expression of TF in leukemia cells or cytokine-stimulated vascular endothelial cells and monocytes. Since RAs and D_3 are similar in their mechanisms of action, we also investigated the anticoagulant effects of D_3. D_3 and its analogs downregulated TF and upregulated TM expression in monocytic cells, counteracting the effects of TNF and oxidized LDL. Pathogenic expression of TF mRNA in monocytic cells was markedly downregulated by D_3 and its synthetic analogs. We have recently found that D_3 suppresses basal and TNF-induced TF expression in monocytic cells by inhibition of AP-1 and NF-□B activation pathways. D_3 analogs also effectively downregulate TF in several cancer cells. The more potent D_3 analogs, which have far stronger binding affinity to D_3 receptor (VDR) have been synthesized.
Conclusions: Several studies report that the D_3/VDR system has a physiological role *in vivo* in the maintenance of antithrombotic homeostasis. We propose that synthetic retinoids and D_3 derivatives could be developed as a new type of antithrombotic agent, which will ameliorate the procoagulant character of abnormal cells and act as antineoplastic agents.

Effects of Intracarotid Injection of Methylprednisolone on Cellular Oedema after Osmotic Opening of the Blood-Brain Barrier in Rats

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Background: In our work we studied methylprednisolone (MP) for its effects on the permeability of cytoplasmatic membranes of neuronal populations in the rat.
Methods: We used a standard model of cellular oedema induced by water intoxication, applying MP selectively into the internal carotid artery(ICA) after opening the blood-brain barrier (BBB) with mannitol. The results were assessed under fluorescence microscopy in keeping with the Intracellular Distribution Index of Evans Blue (IDI EB) in the neocortical field (Cortex) and in hippocampal areas CA1, CA3 and GD (gyrus dentatus). [The IDI EB values range from 0 to 2. IDI EB=1 matches equal extra/intra cellular EB distribution, IDI EB>1 means dominant intracellular EB distribution, IDI EB<1 means dominant extracellular EB distribution]. Evans blue (EB) was applied similarly as MP. Three different experiments were carried out. In experiment 1 - EB alone and no MP was applied. In experiment 2 - 5.4 mg/kg MP and EB were applied. In experiment 3 - 54 mg/kg MP and EB were applied.
Results: In experiment 1 the IDI values were high (>1), indicating the presence of large quantities of EB in the cells. In experiments 2 and 3 the IDI values were low (<1), indicating more EB outside than inside cells. IDI differences between experiments 2 and 1 and experiments 3 and 1 were statistically significant ($p<0.05$).
Conclusions: In our view, this amounts to morphological evidence of cell membrane integrity restored under the effect of MP.

Chlorhexidine Gluconate - Local Antimicrobial Agent in Aid of Prevention and Treatment of Periodontal and Peri-implant Diseases

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Background: Chlorhexidine gluconate (CHX) is the most effective oral antimicrobial and anti-plaque agent and it is the first drug of choice in aid of prevention and treatment of periodontal and peri-implant pathology in clinical situations, mostly when mechanical oral hygiene is compromised or impossible.
Therefore, the aims of our two separated studies were:
1. To evaluate the influence of CHX on the process of wound healing **2.** To assess the substantivity of CHX to titanium surfaces.
Methods: 1. Excisional wounds (5 mm in diameter) were made in the center of the palate of 125 Wistar male rats. CHX, phenolic compounds, amine/stannous fluoride solutions and saline as a control, were applied daily for 1 minute. Six animals were sacrificed from each group at 3, 7, 14 and 21 days post-operatively. Wound areas were measured photographically. The epithelialization rate was determined histologically.
2. Saliva coated machined (M), and sandblasted and acid-etched (SLA), titanium discs were soaked in CHX solution. The levels of adsorbed and desorbed CHX were measured spectrophotometrically and bacterial inhibition on *S. mutans* bacterial lawns were calculated.
Results: 1. The mean area of wounds decreased significantly with time ($p<0.001$) in all experimental and control groups with CHX and phenolic compounds solution presenting the best rate of epithelialization (-10.56 SE 11.05 and -10.06 SE 6.80 respectively).
2. Of the available CHX, 3-8% was adsorbed, SLA surface adsorbed significantly more CHX than M surface ($p<0.001$) and discs immersed in 0.2% CHX adsorbed significantly higher levels of CHX as compared to 0.1% solutions ($p<0.001$). A part of the CHX adsorbed to SLA and M discs (0.6 % and 1.1% accordingly) was desorbed following 24h.The levels of desorbed CHX were significantly affected by CHX concentration ($p=0.013$).The antimicrobial assay revealed that 0.2% CHX solution and SLA discs presented a significantly higher inhibition level than 0.1% CHX and M discs.
Conclusions: 1. Use of CHX as local antimicrobial agent do not impair wound epithelialization and may be used for post operative infection control.
2. The demonstrated evidence of substantivity of CHX to saliva-coated titanium surfaces supports its use onto implant surfaces.

Nifedipine, the most citated, studied, experienced and debated ca-antagonist: where has it gone?

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Of all dihydropyridine Ca-antagonists, that have been introduced since the 1960s, nifedipine was not only the first, but probably since that time, the most prescribed, debated and studied Calcium antagonist. Introduced as the third possibility to treat patients with anginal discomfort, after nitrates and betablockers, it was very much appreciated at that time. Not having the possibility to treat patients with PCI and or routine CABG, adding medication to triple treatment on the coronary care dept. was a standard treatment in unstable or worsening angina, however often proved to work insufficiently for those patients. The first formulations of Nifedipine - short acting – had to be administered 4 times a day, causing massive fluctuations in plasma levels during the day. These short-acting formulations were far from ideal and in 1996 several studies, involving various dosing regiments (up to 120mg/day!) reported possible dangerous effects in secondary prevention. The reports given by Furberg (1995) caused an intensive debate on the safety of these drugs both in the treatment of angina pectoris as in the treatment of hypertension. Following this debate and with the knowledge of the farmacokinetic profile, long-acting formulations of the drug were developed and studied in large scale randomised controlled trials, both in hypertension and angina pectoris. The results of these trials, presented in the period 2000 – 2004 demonstrated both the safety and the positive effect of long acting formulations of Nifedipine, especially the GITS formulation.
As a consequence of these results, guidelines for both hypertension and angina pectoris have recently been reconsidered, and have put the modern, long-acting formulations of the ca-antagonists in a pole position. Within this group of therapeutics, Nifedipine GITS has a unique position and cannot be replaced by just an other Nifedipine formulation, which has not been proven effective and safe in large clinical trials in order to prevent the history to repeat itself.

Fusion Proteins For Flexible Vaccine Antigen Targeting To Cell Surface Receptors

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Background: The initiation of an immune response requires that relevant antigens gain access to the appropriate intracellular compartments to be broken down to peptides, which can then be loaded on major histocompatibility complex (MHC) class I and/or class II molecules. Peptide presentation by cells providing efficient costimulation is important for priming and boosting T cell responses, a requirement met best by dendritic cells (DCs). Targeting of protein vaccines to antigen presenting cells is therefore an attractive strategy for eliciting cellular immune responses. However, because of the difficulty of coupling frequently hydrophobic proteins to targeting devices such as antibodies, its potential has been little exploited. This problem could be bypassed by using fusion proteins.
Methods: We have produced several antigens, including hydrophobic viral proteins with an interest as vaccines, as fusion proteins. The soluble proteins also comprise protein G domains for binding to immunoglobulins, and ubiquitin for proteasome targeting in cross-presentation pathways. The behavior of the fusion proteins has been analyzed *in situ* by fluorescence microscopy. The humoral and cellular immune response of immunized mice has been evaluated by Enzyme-linked immuno-sorbent assay and flow cytometric analyses.
Results: Complexes between fusion proteins and suitable antibodies bind specifically to DCs and are internalized into endolysosomal compartments. The fusion proteins are rapidly transported *in vivo* to CD169+ and later to CD35+ cells in the draining lymph nodes after subcutaneous immunization. Using the model antigen ovalbumin, we show that the fusion proteins, when coupled to various antibodies, elicit a humoral immune response and both MHC class I and class II restricted T cell responses *in vitro* and *in vivo* with an at least 100-fold greater efficiency than antigen alone. Multiple-cytokine-producing CD8+ effector T cells are generated.
Conclusions: 1) The results demonstrate the potential of the strategy for vaccination by initiating a potent immune response. 2) This new tool can be used to stimulate the immune system in a suitable way by targeting the antigen to specific cell surface receptors. 3) A major advantage of this strategy is the possibility to easily compare immune responses by simply exchanging the targeting antibody.

The impact of interferon-β treatment on the blood-brain barrier

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Molecular alterations at the blood-brain barrier (BBB) are essential in the pathogenesis of multiple sclerosis (MS). The implementation of the immunomodulators as the first evidence-based treatment option in the mid 1990s opened a new era in multiple sclerosis therapy.
Interferon-β (IFN-β) is commonly suggested to act as an immunomodulator of the cytokine network. However, there is increasing evidence that IFN-β leads to a stabilization of BBB integrity in MS patients. This paper will show recent advances in MS with focus on the BBB and will present the author's contribution on revealing direct and indirect effects of IFN—β on the BBB. It will summarize recent work both with MS patients as well as experimental *in vivo* and *in vitro* data.
The understanding of IFN-β-derived stabilization of the BBB will not only provide new insights in the pathogenesis of MS but also might be helpful in the development of new, more specifically designed drugs in the treatment of MS.

Effects of Small-volume Resuscitation Using Hyperosmolar Saline Colloid Solution on Regional Blood Flow and Ischemic Tissue Injury

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Background: The concept of Small-volume Resuscitation (SVR) – the rapid infusion of a small dose (4 ml per kg B.W.) of 7.2 – 7.5% NaCl/colloid solution – has been advocated for initial therapy of severe hypovolemia and shock. The solution seems to encompass specific pharmacologic effects, highly relevant for prevention of multiple organ dysfunction syndrome (MODS) induced by trauma and shock.
Methods and Results:
- Hemorrhagic shock
Using the radioactive microspheres method we demonstrated the positive circulatory effect of 7.2% saline given in an amount as small as 1/10 of actual blood loss. In a standardized animal model of severe hemorrhagic hypotension in dogs macrohemodynamic parameters and blood volume were normalized and nutritional blood flow (RBF) recovered within 5 min. RBF was completely restored when combining 7.2% saline with 10% dextran 60 (HHS; hyperosmolar-hyperoncotic solution).
- Endotoxin shock
In a pig model of endotoxin shock elicited by continuous i.v. infusion of *S. abortus equi* leading to a hyperdynamic state with severe circulatory and pulmonary deterioration, 4 ml/kg B.W. of HHS led to significant enhancement of RBF particularly in the small intestine and kidneys.
- Reperfusion injury
Using the hamster dorsal skin-flap for analysis of leukocyte-endothelial interaction HHS proved to ameliorate the activation of polymorphonuclear neutrophils (PMNL). Functional capillary density was augmented and extravasation of macromolecules diminuished.
- Up-regulation of beta2-integrins
In *in-vitro* experiments, hyperosmolar saline attenuated N-fomyl-methionyl-leucyl-phenylalanine (fMLP) stimulated expression of adhesion molecules on PMNLs. fMLP-stimulated up-regulation of beta2-integrins was diminished.
- Subarachnoid hemorrhage
In a standardized model of subarachnoid hemorrhage (SAH) in rats treatment with 7.5% NaCl plus 6% dextran 70 resulted in lowered intracranial pressure, improved neurological recorery and less morphological damage.
Conclusions: 1) Small-volume hyperosmolar colloid resuscitation from severe hemorrhage and shock rapidly mobilizes endogenous water. 2) Nutritional blood flow is enhanced and reperfusion injury diminuished. 3) Following subarachnoid hemorrhage hyperosmolar saline dextran solution improves neurological outcome.

Anti-Idiotypic Vaccines Against Autologous Antigens: Designed Ankyrin Repeat Proteins as Scaffolds to Break Self Tolerance?

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According to the network hypothesis antibodies may act as antigens to induce an anti-idiotypic immune response. The binding site of an anti-idiotypic antibody thus represents the internal image of an epitope present on the original antigen. When formulated as a vaccine, it has been recently demonstrated that anti-idiotypic antibodies or antibody fragments may be used to regulate the immune responses associated with autoimmunity, cancer or allergy. In allergy, binding of IgE to its high affinity receptor (IgERI) is a key pathogenic event. A humanized monoclonal anti-IgE antibody (Omalizumab, Xolair®) which inhibits the IgE / IgERI interaction has been developed for the treatment of severe allergic asthma. Recently we described the isolation of anti-idiotypic antibody fragments specific for the omalizumab-like anti-IgE antibody BSW17 from a non-immune human Fab phage display library. Immunization of rabbits with these Fabs induced a neutralizing antibody response against human IgE. Novel binding molecules based on designed ankyrin repeat proteins (DARPins) may serve as an alternative to antibodies with improved potency to break self tolerance. Using a consensus DARPin library design strategy employing varying repeat numbers and randomized surface residues, anti-idiotypic binders against mAb BSW17 were identified which are now being further evaluated as putative anti-idiotypic vaccine candidates.

Molecular Changes Induced by Stress Factors in Cerebral Endothelial Cells

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Being located at the interface of blood and brain, cerebral endothelial cells (CECs) are primary targets of different environmental stimuli.
The aim of our study was to reveal molecular mechanisms activated by oxidative, hyperosmotic, and calcium depletion induced stress in CECs using an in vitro model of the blood-brain barrier (BBB).
There is increasing evidence that the cerebral endothelium and the BBB play an important role in the hypoxia induced brain damage. We have shown that oxidative stress induces a downregulation of the tight junction protein occludin which is more pronounced in the absence of glucose. Furthermore, oxidative stress leads to disruption of the cadherin - catenin complex and an activation of ERK1/2, which is more intense in the absence of glucose. These results indicate that one of the causes of the BBB breakdown is the structural alteration of the junctional complex caused by oxidative stress, a process in which ERK1/2 may play an important role.

Hyperosmotic stress elicited by mannitol has been successfully used to reversibily open the BBB. We have shown that hyperosmotic conditions induce protein phosphorylation on both Ser/Thr and Tyr residues. Among the targets of protein tyrosine phosphorylation is the adherens junction protein beta-catenin. Phosphorylation of beta-catenin on tyrosine residues caused its subcellular redistribution and its dissociation from cadherin and alpha-catenin. All these effects were Src kinase dependent. Osmotic stress is able to induce tyrosine phosphorylation of Axl followed by activation of Akt as well. Moreover, Axl was also cleaved in response to osmotic stress resulting in a 50-55 kDa double degradation product. This process was mediated by a metalloproteinase-dependent cleavage, followed by a proteasomal cleavage.
We have shown that besides changes in junctional protein expression and localization calcium removal induces significant changes in the morphological parameters of CECs as well as revealed by atomic force microscopy. These changes could be partially inhibited by the Rho-dependent kinase inhibitor Y27632 suggesting a role for ROCK in mediating the effect of low calcium concentration in CECs.
Our results show that multiple signaling pathways are activated by different stress factors in CECs.

Pharmacokinetic and Pharmacodynamic Modeling of Recombinant Human Erythropoietin.

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Erythropoietin is a hematopoietic growth factor stimulating the production of RBC. Recombinant human erythropoietin (rHuEPO) has been indicated for treatment of anemias associated with renal failure, cancer chemotherapy, and HIV antiviral therapy. rHuEPO is an alternative treatment for blood transfusions and its clinical endpoint is to elevate hemoglobin levels. The major clearance mechanism for rHuEPO is binding to its receptor (EPOR) followed by internalization and degradation. EPOR are mostly expressed on bone marrow erythroid progenitor cells. Upon binding to its receptor rHuEPO initiates several intracellular signaling pathways leading to inhibition of cell death, increase in proliferation, and acceleration of the differentiation processes. All of which causes an increase in number of progenitor cells in bone marrow, and reticulocytes and RBC in blood. There will be presented several pharmacokinetic (PK) and pharmacodynamic (PD) models that have been developed to describe serum rHuEPO concentrations and reticulocyte, RBC, and hemoglobin responses obtained from clinical trials and studies in animals. The focus will be on applications of concepts target-mediated disposition and lifespan based indirect response in PK/PD modeling of rHuEPO. Topics will encompass the nonlinear structure of presented models and stress usefulness of mathematical models in assessment of rHuEPO efficacy and potency. Conclusions will include possible modifications of the PK/PD models to account for neglected so far processes and applications of existing models in development of new protein drugs exhibiting a similar to rHuEPO mechanism of action.

Thiopurine *S*-methyltransferase and inosine triphosphate pyrophosphohydrolase genes in Japanese patients with inflammatory bowel disease in whom adverse drug reactions were induced by azathioprine/6-mercaptopurine treatment

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Background: The thiopurine *S*-methyltransferase (TPMT) and/or inosine triphosphate pyrophosphohydrolase (ITPA) gene mutations are suggested to be closely related to adverse reactions induced by azathioprine (AZA)/6-mercaptopurine (6MP) in Japanese. Screening for mutant alleles may be useful for predicting development of the most serious adverse reactions, agranulocytosis and acute bone marrow suppression.
Methods: Gene mutations for TPMT and ITPA, major AZA/6-MP-metabolizing enzymes, were investigated in 103 healthy Japanese (45 males, 58 females; mean 32.1±8.35 years old) and 16 Japanese patients (9 males, 7 females; 39.1±15.4 years) with inflammatory bowel disease, in whom AZA/6MP treatment induced adverse reactions. *TPMT*2* (238g>c) and **3C* (719a>g) were analyzed using an allele-specific PCR method, and the *TPMT*3A* (460g>a, 719a>g), **3B* (460g>a) and ITPA 94c>a mutations by PCR-RFLP. All experiments were approved by the Ethics Committee of Jikei University School of Medicine.
Results: Analysis of ITPA genes in the 103 healthy subjects showed that 75 (72.8%) were wild-type, while 25 (24.3%) were heterozygous mutations, and 3 (2.9%) homozygous mutations. The frequency of the 94c>a mutant allele was 15% (0.150, 95% confidence interval: 0.108-0.206). The TPMT gene was the wild-type in all 16 patients, whereas the overall ITPA gene 94c>a mutation detection rate was 50%, with rates of 83.3% in patients with acute bone marrow suppression and 75% in those with agranulocytosis. The 94c>a allele frequency was 31% (0.313, 95% confidence interval: 0.180-0.486), which was significantly higher than the Japanese standard (*chi*-square test, $p<0.05$), while adverse reactions developed earlier in patients with that mutation. In half of the patients, no gene polymorphism was noted, suggesting involvement of drug interactions and reduction of TPMT activity.
Conclusions: In predicting thiopurine adverse reactions in Japanese, it may be favorable to perform screening for the 94c>a mutation, followed by determination of *in vitro* TPMT activity when the gene is the wild-type to investigate drug interactions.

Memorabilia of Paul Ehrlich in modern Strzelin (Strehlen) and Wroclaw (Breslau).

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Background: Paul Ehrlich (1854-1915), the best known for discovery the idea of chemotherapeutics, as he expressed it “magic bullets”, chemical substances that have a specific affinity to pathogenic microorganisms, and can destroy them, was given a Nobel prize in 1908. We wish to commemorate Paul Ehrlich on the centennial of his Nobel Prize in Physiology or Medicine in 1908 as he is the most famous person born in *Strehlen*, now *Strzelin*, little city in downer Silesia, region where our Medical University is located.
Methods. Descriptive study; we visited *Strzelin* and *Wroclaw* tracing memorabilia of Paul Ehrlich. Photographic and written documentation was made.
Results. *Strzelin* (to 1945 *Strehlen*) is now a little calm town located at Olawa river with 13.300 inhabitants. Ehrlich’s family house situated at the market square was destroyed during second world war in 1945 and a pharmacy building occupies this place now. There is an monument commemorating Paul Ehrlich at the crossing of present *Dzierżoniowska* and *Bolka I Świdnickiego* streets founded in 2004. Wroclaw (to 1945 Breslau) with about 630 000 inhabitants is rapidly growing modern city, the fourth largest in Poland. It is the capitol of downer Silesia Province. Paul Ehrlich finished St. Magdalene Gimnasium located on present *Szewska* street (former *Schubrücke*) near St Magdalene Church in 1872. The gymnasium founded in 1643 was completely destroyed during second world war in 1945. Then Paul Ehrlich studied medicine at the Breslau University. The University (*Schlesische Friedrich-Wilhelm-Universität zu Breslau*) founded on 3 August 1811 by merging of Leopoldinum Academy dated from 1702 with Viadrinia University established in 1506 in Frankfurt upon Oder and included 5 faculties: catholic theology, evangelic theology, law, medicine and philosophy at his time. At present Wroclaw University (*Uniwersytet Wrocławski*), its direct successor educates over 38.000 students and consists of 10 faculties.

Tolerability of pirymethamine/sulpha chemotherapy in children with congenital toxoplasmosis treated in Department of Pediatrics and Infectious Disease, Medical University in Wroclaw, Poland.

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Background: Toxoplasmosis is a wide-spread protozoan diseases occurring in congenital and acquired form. Congenital infection with rate reaching 1 per 1000 of newborns may involve the retina and brain with long-life sequellae. Despite the discovery of new antibiotics combined therapy with pirymethamine/sulpha has been the first line treatment for congenital toxoplasmosis for more than 20 years. The aim of the study was to sum up single centre experience with tolerability of the treatment in last 8 years.
Methods. Descriptive retrospective study; 12 children with congenital toxoplasmosis treated with standard doses of pirymethamine/sulpha and folinic acid supplementation in the Department of Pediatrics and Infectious Diseases in Wroclaw, Poland between 2000 and 2007 were enrolled. Disease was diagnosed with serologic tests in all patients but one, in whom the diagnosis was made by PCR.
Results. 12 children aged from 5 days to 3 years (8 girls and 4 boys) were treated for 3 weeks to 13 months (median 9 months). We observed thrombocytopenia in 3/12, anemia in 3/12, leucopenia in 2/12, cholestasis in 2/12 and elevated aminotransferases in 1/12 treated children respectively. The therapy had to be suspended for 4 to 6 weeks in 3/12 children due to thrombocytopenia mainly. Therapy was effective in all treated children with 100% survival in a follow up ranging from 1 to 8 years.
Conclusions. Chemotherapy of congenital toxoplasmosis with pirymethamine/sulpha and folinic acid supplementation in infants is effective and generally well tolerated. The most common undesirable effects include brainstem toxicity and need regular complete blood count monitoring.

Benzimidazoles and surgery in cardiac hydatidosis: efficacy in prevention of disease relapse

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Background: Echinococcosis is a pathology encountered in animal husbandry countries. Cardiac echinococcosis (CE) is a rare entity that might cause serious life-threatening complications. Management strategies include surgical removal (cystectomy) of hydatid cysts and adjunctive benzimidazole derivatives therapy (BDT). However, there are no randomized trials on the recurrence rate of CE in patients treated with BDT therapy as adjunct to surgery.
We aimed to analyze case series on surgical correction of CE with adjunctive BDT reported in literature and own experience to define the recurrence rate in such combinative treatment and analyze cases with recurrence of disease.
Methods: We made search in MEDLINE using MESH terms – “cardiac echinococcosis” and “heart diseases” for a period between 1998 and 2008 years. Overall, there were 166 articles retrieved, among them 14 were reporting series (2 to 62) of patients (pts) treated with surgical correction and BDT.
Results: Fourteen studies reported included overall 164 pts with CE (age range 9-75 years, and 88 pts were female). The cyst localization was – left ventricular (LV, including interventricular septum (IVST) aspect) – 37.1% (61 pts, including IVST aspect), right ventricular (RV) – 18.9% (31 pts), IVST –17.6% (29 pts), right atrial – 9.1% (15 pts), left atrial -1.8% (3 pts), pericardium – 9.1% (15 pts), sinus of Valsalva – 0.6% (1 pt); 5 pts (3%) had cysts in LV and pericardium, 4 pts (2.4%) – RV and pericardium. Of 164 pts 40 (24.3%) pts had associated lesions in liver (17 pts), lungs (12 pts), spleen (2 pts), and aorta (1 pt). Multiple lesions were found in 8 pts: brain, spine and spleen – 1 pt, brain and kidney – 1 pt, liver and lung - 4 pts, liver and spleen – 1 pt, liver, lung and brain – 1 pt.
Surgical removal of hydatid cysts was performed using off-pump cardiac surgery in 47 pts (28.7%) and using cardiopulmonary bypass – 117 pts (71.3%). Therapy with albendazole was started in 81 pts, with mebendazole – in 29 pts. The follow-up period varied between 3 months to 12 years. The CE recurrence rate for albendazole was 3.7% (3 of 81 cases), and 3.4% for mebendazole (1 of 29 pts). The CE relapse occurred in pts taking albendazole with multiorgan, multiple and complicated lesions (ruptured cysts). Few pts were taking albendazole prior to operation (2 weeks-12 weeks) due to previous lesions in other organs. Cysts characteristics for these pts were distinctive by their solid, unviable and inactive nature, posing less complicated removal during cardiac surgery as compared with cysts at active stage, which might have risk of rupture and dissemination of daughter cysts during surgery.
Conclusion: 1. BDT as adjunct to surgery for cardiac echinococcosis is accompanied by recurrence rate of 3.7%. 2. The CE relapses are related to multiple damage, rupture of cysts and complicated course of disease. 3. Albendazole taken prior to surgery might facilitate the successful surgical removal of cardiac cysts.

Towards a Magic Bullet for the Metabolic Syndrome – Use of the Ginkgo Biloba Extract

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Background: The metabolic syndrome represents a collection of inter-related metabolic defects commonly associated with diabetes (e.g. dyslipidemia, obesity and hypertension) that increase the risk of cardiovascular disease. Ingestion of the dietary supplement *Ginkgo biloba* Extract might ameliorate the metabolic syndrome by reduction of oxidative stress in blood plateletes and the normalizationation of pancreatic beta-cell function in especially T2DM patients with pancreatic exhaustion. Since hyperinsulinemia is a hallmark of T2DM, it is important to verify that increased insulin production is not due to increased insulin resistance. The primary aim is to measure the effect of the ingestion of *Ginkgo biloba* extract on whole body insulin sensitivity in subjects with normal glucose tolerance (NGT), impaired glucose tolerance (IGT, pre-diabetes) and full-blown T2DM.
Methods: Subjects with NGT (n = 10; age, 44.2 ± 13.9 years old), impaired glucose tolerance (IGT) (n = 8; age 51.3 ± 6.6 years old) and T2DM (n = 8, 51.6 ± 15.2 years old) completed a randomized, double-blind, placebo-controlled crossover study, with each arm lasting 3 months. After ingestion of either *Ginkgo biloba* extract (120 mg/day as a single dose) or placebo for each arm, a 2-step (each lasting 2 hours) euglycemic insulin clamp was performed, using [^{3}H]glucose intravenous influsion and whole body glucose metabolic rate (M-value) was calculated during the last 30 min of each step.
Results: At the low insulin infusion rate (10 mU/m^2/min) the glucose metabolic rates (M values) were 3.5 ± 1.5 vs. 3.0 ± 0.5 mg/kg (P = 0.16), 3.0 ± 0.4 vs. 2.8 ± 0.8 mg/kg (P = 0.19) and 2.6 ± 0.7 vs. 2.4 ± 0.5 mg/kg (P = 0.09) for the placebo and *Ginkgo biloba* cycles, in the NGT, IGT and T2DM subjects, respectively. At the high insulin infusion rate (40 mU/m^2/min) the M values were 7.3 ± 2.3 vs. 8.1 ± 2.5mg/kg (P = 0.07), 6.2 ± 1.6 vs. 6.5 ± 2.1 mg/kg (P = 0.32) and 3.6 ± 1.6 vs. 3.5 ± 1.0 mg/kg (P = 0.34) for placebo vs. *Ginkgo biloba* cycles, in the NGT, IGT and T2DM subjects, respectively.
Conclusions: The ingestion of 120 mg of *Ginkgo biloba* extract as a single for 3 months had no significant effect on insulin resistance in non-diabetic subjects or those with pre-diabetes (IGT) nor did it exacerbate the disease in those with full-blown T2DM. Considering the other benefits of ingesting *Ginkgo biloba*, this is as close to a magic bullet as there is fort he metabolic syndrome.

Acknowledgement: This study was supported by funds from NIH grants M01-RR-01346 and R01-AT-00832 (National Center for Complementary and Alternative Medicine, NCCAM).

Experimental Evidence for Killing the Resistant Cells and Raising the Efficacy of Cytostatics by a New Anticancer Drug Candidate (Culevit Infusion) Developed on the Basis of the Passive Antitumor Defence System

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Background: While other research groups, realizing the inefficiency of the immune system against cancer, studied immune escape or response modifiers we thought that the failure of tumours to develop in the majority of the population during their lifetime despite ineffective immune surveillance indicates the existence of other defence system(s) whose agents should lie in the circulatory system (CS). It is well known and used for tumour detection (as in PET) that the uptake of most substances (amino acids, monosacharides, nucleobases, etc.) occurring in CS is strictly regulated by normal cells, whereas it is unregulated and elevated by tumour cells. Considering that many molecules in the living system have more than one role we supposed that some of the accumulated substances might be the agents of a defence system capable of killing emergent cancer cells. We substantiated this hypothesis by experimentally selecting 16 substances of CS (of 89 investigated) whose mixture had toxic effects in vitro and in vivo on all tumour cell lines investigated, but not on normal cells and animals. Knowing the 16 agents protected by patents in many countries, the development of a medicine in an infusion form is at the preclinical phase.
Methods: The infusion was administered i.p. as injection 8 times a day for 10 days.
Results: Significant inhibitory effects of the infusion have been observed in the case of all tumours (P-388, S-180, B-16, MXT, Colon-26, He/De, Ne/De, HL-60) investigated. The infusion compared to cytostatics (5-FU, Cisplatin) had slightly better inhibitory effect on Colon-26. It is important to note that the infusion significantly increased the effects of both cytostatics when they were used simultaneously. It appeared that the components of the infusion could also kill multidrug resistant cells (AT3B-1, MCF7/ADR) and the infusion abrogated the resistance towards 5-FU of B16 melanoma. No lethality, toxic effects, adverse clinical symptoms were observed in toxicity studies.
Conclusion: The Culevit infusion, besides having significant inhibitory effect on tumours, can improve the efficacy and reduce the side effects of chemotherapy and radiation and can decrease the possibility of relapse.

***In silico* Approaching to Cisplatin Toxicity**

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Background: A new approach to evaluation of the mode of action in platinum anticancer drugs arena are quantum chemical in silico studies which have, so far, focused on cisplatin hydrolysis and then binding to purine bases of DNA. These reactions are believed to be the basis of anticancer activity of cisplatin and its analogues. Contrary this, we concentrated on the toxicity of cisplatin, particularly in comparison with non-toxicity of its trans isomer. Thus the impact of platinum(II) on sulphur-containing molecules, present in serum, was the object of this study. In serum, because of the high chloride concentration, cisplatin and transplatin are found in chloro- form. Aim: theoretical studies on reactions of non-hydrolyzed (NH3)2PtCl2 with thiols.
Methods: The following systems were investigated: (1) cisplatin (transplatin) with CH_3SH; (2) cisplatin (transplatin) with L-cysteine. The electronic structure for molecular systems was studied at non-empirical all-electron level by using density functional (DFT) or Moeller-Plesset (MP2) methods within the correlation consistent cc-pVTZ basis set. In the case of platinum the widest Huzinaga basis set with polarization functions was used. At the first stage, the optimization was performed at the all-valence MOPAC-PM6 method following the B3LYP density functional or MP2 formalism in the next step. The B3LYP density functional was applied using GAUSSIAN-03 program package. The numerical calculations have been performed in part at Wroclaw Networking and Supercomputing Center.
Results: The order of reactivity in the impact of cysteine on Pt(II) was: transplatin (PCM)> cisplatin (PCM) > transplatin (gas phase) > cisplatin (gas phase);
The order of reactivity in the impact of CH_3SH on Pt(II) was: transplatin (gas phase) > transplatin (PCM) . > cisplatin (PCM) > cisplatin (gas phase)
Conclusions: (1) Quantum chemical in silico methodology was applied to new evaluation of energy interaction in P(II) – thiol systems. (2)The established rows of reactivity showed good agreement with results of earlier biochemical and kinetic studies.

New Animal Models for Psychotropic Drug-Drug Interactions

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Background: The involvement of brain neurotransmitters and trophic factors in the mechanisms of psychotropic drug action is commonly accepted. The existent animal models are based on the effects psychotropic drugs on the behavior induced with chronic stress or knockout of the genes involved in the regulation of brain neurotransmission. Here new models for study the role of serotonin and cytokines in psychotropic drug-drug interaction were presented.
Methods: The three new genetic models were created: 1) the ASC/Icg (Antidepressant Sensitive Catalepsy) mouse line selectively bred from a backcross population between CBA/Lac and AKR/J strains for high predisposition to catalepsy; 2) the AKR.CBA-D13Mit76 congenic mouse line with the 61-70 cM CBA-derived fragment of chromosome 13 transferred to the AKR genome (Kulikov et al., Genes, Brain Behav., 2008, 7:506-512); and 3) the B6-1473G congenic mouse line with the 1473G allele of the tph2 gene decreasing activity of the rate-limiting enzyme of serotonin synthesis in the brain, tryptophan hydroxylase-2, transferred to the C57BL/6J genome.
Results: The major gene defining predisposition to catalepsy was mapped on the 61-70 fragment of mouse chromosome 13 and linked to the Il6st gene coding the gp130 protein associated with cytokine receptors. The ASC mice showed numerous depressive-like traits and altered serotonin neurotransmission compared with the parental CBA and AKR strains. Chronic antidepressant treatment decreased catalepsy in ASC, but did not affect the trait in CBA mice. The transfer of the 1473G allele of the tph2 gene to the C57BL/6 genome significantly affected the intermale aggression and depressive-like immobility in the forced swim test.
Conclusion: 1. The ASC mouse line meets face, predictive and construct validity criteria of animal model of depression and antidepressant drugs screening.
2. The AKR.CBA-D13Mit76 congenic mouse line with altered gp130 protein is a promising model to study the interaction between cytokines and psychotropic drugs.
3. The B6-1473G congenic mouse line with altered tph2 gene is a valuable model of the interaction between serotonin and psychotropic drugs.

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Anticancer activities of vitamin D analogs

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Background: *All-trans*-retinoic acid (ATRA) is used for the treatment of acute promyelocytic leukemia as a non-chemotherapeutic drug that induces terminal differentiation of leukemia cells. Another seco-steroid, $1,25(OH)_2$ vitamin D_3 [$1,25(OH)_2D_3$] and related compounds also have anti-cancer activities against various types of cancer cells by inhibiting proliferation and inducing differentiation of tumor cells *in vitro and vivo*. Although orally administrated $1,25(OH)_2D_3$ had modest usefullness for patients with myelodysplastic syndrome (MDS) in clinical studies, its use was hampered because of hypercalcemia. 19-nor-$1,25(OH)_2D_2$ (Paricalcitol) is approved by the FDA for the clinical treatment of secondary hyperparathyroidism in patients with chronic renal failure. Different from other vitamin D analogues, paricalcitol has very little calcemic potential. This prompted us to investigate its anti-proliferative effect against cancer cells.
Methods: We studied anti-proliferative effect of paricalcitol against cancer cell lines in vitro and in vivo. The combinations of the analog with other clinally useful agents were also tested in vitro.
Results: Paricalcitol has antiproliferative effects against human cancer cells including prostate and colon cancer cells, as well as leukemia and multiple myeloma cells by inducing differentiation, cell cycle arrest and apoptosis *in vitro* and *in vivo*. Among many combinations with other clinically useful agents tested, paricalcitol in combination with arsenic trioxide has markedly enhanced antiproliferative activity against acute myeloid leukemia cells including acute promyelocytic leukemia cells. In further studies, arsenic trioxide acts as an inhibitor of both 24-hydroxylase which is a negative feedback regulator of vitamin D and the PML-RARalpha, the leukemogenic fusion protein in vitro. This may explain the synergistic effect of the combination against myeloid leukemia cells.
Conclusions: The hypercalcemic side-effect of $1,25(OH)_2D_3$ has been mitigated by less-calcemic vitamin D analogs such as paricalcitol. The analog and its combination with other clinically useful drugs are being investigated with the hope that they may provide a therapeutic approach to cancers with little toxicity.

Purification and properties of a chemotherapeutic enzyme, L-asparaginase, from *Pectobacterium carotovorum* MTCC 1428.

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Background: L-asparaginase is widely used in the chemotherapy. However, the success hitherto has been rather limited and most of the treatments have not been so successful due to various allergic reactions. Some of allergic reactions are mainly due to glutaminase contamination of most L-asparaginase. Hence, the discovery of a glutaminase-free L-asparaginase isolated from an organism that is serologically different from the previously reported ones, but has similar therapeutic effects will be more advantageous. In this communication we report on the purification and properties of glutaminase-free L-asparaginase extracted from *P. carotovorum* MTCC 1428.
Methods: The production of L-asparaginase from *P. carotovorum* was studied in the modified M-9 medium at 30°C and 180 rpm for 12 hrs. The harvested cells were ultrasonicated and centrifuged at 20000g for 20 minutes at 4°C to obtain crude extract of L-asparaginase.The purification was carried out by ammonium sulfate fractionation (80% saturation), DEAE cellulose ion exchange chromatography and Sephadex G-100 gel chromatography. The various purification steps were examined using Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) following the method of Laemmli. A Lineweaver-Burk analysis was used to determine kinetic properties of the purified L-asparaginase.
Results: The L-asparaginase was purified to homogeneity from *P. carotovorum* cells. Different purification steps (including ammonium sulfate fractionation followed by separation on DEAE cellulose chromatography and Sephadex G-100 gel filtration) were applied to the crude culture filtrate to obtain a pure enzyme preparation. The enzyme was purified 70-fold and showed a final specific activity of 1952 IU/mg with a 39% yield. SDS-PAGE gel showed a single protein band after sephadex G 100 gel filtration which revealed the purity of L-asparaginase. K_m and V_{max} values of purified L-asparaginase was compareable with the existing one which is used as a drug.
Conclusions: This work gives promising results on the possible production of glutaminase-free L-asparaginase which is particularly important for the development of downstream process for efficient production of L-asparaginase from *P. carotovorum* in a large scale.

Resistance, Including Carbapenem Resistance, Among Enterobacteriaceae In a University Hospital In Singapore

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Background: Our study was designed to detect enzymes such as ESBLs and AmpC in health-care associated strains of Escherichia coli and Klebsiella pneumoniae, using phenotypic methods and to further characterize carbapenem resistant isolates.
Methods: To detect enzymes such as ESBLs and AmpC in Escherichia coli and Klebsiella pneumoniae three-dimensional extract (TDE) method was used. For K. pneumoniae and Enterobacter cloacae with reduced susceptibility to carbapenems a phenotypic disk diffusion bioassay and double disc diffusion methods were used to elucidate the presence of multiple ?-lacatamases such as AmpC, MBLs, and carbapenemases.
Results: When testing 126 isolates that were resistant to third generation cephalosporins (39 E. coli and 87 K. pneumoniae) in 2005, we found AmpC in 28.2 % (11 isolates) of E. coli and in 17.2 % (15 isolates) of K. pneumoniae.
In addition 6 isolates were resistant to carbapenems (5 isolates of K. pneumoniae and 1 isolate of E. cloacae)
A combination of phenotypic tests revealed a likely co-existence of AmpC and ESBL in both E. coli and K. pneumoniae. Resistance to cefepime was observed in 83% of phenotypic AmpC positive isolates (10 out of12 tested isolates).
Multiple ß-lactamases were detected in K. pneumoniae and E. cloacae with reduced susceptibility to carbapenems. The presence of carbapenemases was suspected in isolates with reduced susceptibility to carbapenems, using phenotypical methods. A single isolate of K. pneumoniae was suspected to harbour a carbapenemase and a MBL, using phenotypical methods. K. pneumoniae with phenotypic AmpC and carbapenemases were susceptible to amikacin and trimethoprim sulfamethoxazole.
An increase in resistance to ceftazidime in E. coli isolates was observed in the hospital between 2005 and 2007 while a decrease was observed in K. pneumoniae.
Overall resistance against cefepime was 24.6% in 2007 for E. coli and 39.3% for K. pneumoniae. There were no differences in resistance to ceftriaxone and ceftazidime indicating that AmpC carrying isolates are still rare in the hospital population. However, among isolates from both species there was reduced sensitivity to imipenem.
Conclusions: Results of phenotypic tests for resistance properties may be helpful. Further work is being done to compare the results of phenotypic tests with the outcome of genotypic investigations.

Missile Injuries of Orofacial Region, Primary and Secondary Phase Managements

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Background: In a society struggling to rebuild the country after three decades of years of dictatorships and wars, Iraqi craniofacial and maxillofacial surgeons play a critical role in treatment of many most serious missile injuries of orofacial region by on going conflict in Iraq. This study reflect the modern and advanced surgical techniques of treating explosive missile injuries and other combat and terrorism related injuries and also evaluate the immediate phase and secondary phase managements of 167 patients suffering from missile injuries .
Methods: This studies include one hundred sixty seven patients with missile injuries of orofacial region in a period of 4 years, all injured patients were treated in maxillofacial unite,10th floor, surgical specialties hospital, medical city ,Baghdad.Thier were 134 men and 33 women, the age ranged from 9 to 70 years (mean 39.5 years)
Result: In addition to27 patients with orbital injuries, their were 134 patients men and 33 women; their age ranged from9 to70 years (mean 39.5 years).
Orofacial deformities classified into following 1-sixsty patients(35.9%) had bone loss 2-fifty patients(29.94%) had soft tissue loss 3- twenty seven patients(16.16%) with orbital injuries 4- thirty patients(17.96%) had other deformities' of scar contracture, fistula and sinus formation.
The bony defect was reconstructed by both bone chips carried by osteomesh tray harvested from the iliac crest and by block of cortico-cancellous bone graft from the iliac crest. Soft tissue reconstruction done by local flaps and regional flaps such as lateral cervical and cervico-facial flaps and orbit reconstructed by bone graft, lypholised Dura and sialastic implant. Scar contracture treated by scar revision and sinus tract excised at the same time of scar revision.
Conclusions: Primary phase required an urgent airway management, controlling an active bleeding by surgical intervention; most entrance and exit wounds as well as retained missile were located in the cheek, chin and mandibular body with few cases of mortality due to complication related to head injuries. Secondary phase managements of deformities' of the face as a complication of missile injuries were classified as bone loss, soft tissue loss, combined bone and soft tissue loss and others(sinus tracts and poor scars).

Serum hepcidin level (HEPC) is a significant predictor of arterial stiffness in maintenance hemodialysis patients (mHD)

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Background: We have already demonstrated that HEPC were significantly higher in mHD than control. Furthermore, in multiple regression analysis, only ferritin (β=0.514, *F*=75.78, p<0.0001) was selected as a significant predictor of HEPC. Recently, HEPC has been suspected to be linked to the cause of anemia of inflammation and cardiovascular disease (CVD). It has been well known that pulse wave velocity (PWV) is a predictor of CVD. For the purpose of clarifying the relationships among HEPC, iron metabolism and CVD, we evaluated HEPC, indexes of iron metabolism, risk factors of CVD, and brachial-ankle (ba)-PWV in mHD. **Methods:** 198 mHD, who were treated with erythropoietin and 33 healthy controls were recruited in this study. Hemoglobin (Hb), β2-microglobulin (MG), calcium (Ca), phosphorus (P), intact-parathyroid hormone (int-PTH), total cholesterol (T-CHO), triglyceride (TG), iron, HEPC, ferritin, total iron binding capacity (TIBC), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and ba-PWV were also measured. HEPC was measured by liquid chromatography tandem mass spectrometry. **Result:** Serum levels of TNF-α(27.1±0.7 vs 5.3±0.9 pg/mL; p<0.0001) , IL-6 (12.4±1.2 vs 3.6±1.1 pg/mL; p=0.017), and HEPC (44.8±2.9 vs 4.4±3.7 ng/mL; p<0.0001) were significantly higher in mHD than control. ba-PWV was significantly correlated with TNF-α(R=0.25 , p= 0.0072), and HEPC (R= 0.28, p=0.0064), but not with IL-6, duration of HD, Kt/V, Ca, P, int-PTH, β2-MG, T-CHO, or TG. In multiple regression analysis, TNF-α (β=0.28, *F*=7.021, p<0.0001) and HEPC (β=0.26, *F*= 5.98, p<0.0001) were selected as significant predictors of ba-PWV in mHD. **Conclusion;** Both HEPC and TNF-α were significant independent predictors of ba-PWV in mHD. These finding show that iron metabolism and inflammation might affect arterial stiffness, which could link to CVD in mHD.

Presentation of usage of new digital technologies in order to advance scientific research in biochemistry and medicine

KURIC L

Beckground: The modern science mainly treats the biochemical basis of sequencing in bio-macromolecules and processes in biochemistry. One can ask weather the language of biochemistry is the adequate scientific language to explain the phenomenon in that science. Is there maybe some other language, out of biochemistry, that determines how the biochemical processes will function and what the structure and organization of life systems will be? The research results provide some answers to these questions. They reveal to us that the process of sequencing in bio-macromolecules is conditioned and determined not only through biochemical, but also through cybernetic and information principles.
Methods: What we did is the following: We translated the physical and chemical parameters from the language of biochemistry into the digital language of programmatic, cybernetic and information principles. This we did by using the adequate mathematical algorithms. By using chemical-information procedures, we calculated the numerical value for the information content of molecules. What we got this way is the digital picture of the phenomenon of biochemistry. These digital pictures reveal to us a whole new dimension of this science.
Results: Within the digital pictures in biochemistry, the physical and chemical parameters are in a strict compliance with programmatic, cybernetic and information principles. As an example, we will here give you the mathematical gravity forces. These forces determine the positioning of aminoacids in their molecules. Each bar in the protein chain attracts only the corresponding aminoacid, and only the relevant aminoacid can be positioned at certain place in the chain. Each peptide chain can have the exact number of aminoacids necessary to meet the strictly determined mathematical conditioning. It can have as many atoms as necessary to meet the mathematical balance of the biochemical phenomenon at certain mathematical level, etc
Conclusion(s): 1)The process of sequencing in bio-macromolecules is conditioned and determined not only through biochemical, but also through cybernetic and information principles. 2)The digital pictures of biochemistry provide us with cybernetic and information interpretation of the scientific facts. 3)Now we have the exact scientific proofs that there is a genetic language that can be described by the theory of systems, and which functions in accordance with certain principles.

Acridine orange found in Ehrlich's era could become a "Magic Bullet" against cancer under photon energy

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Background: Acridine orange (AO) was extracted as a dye from coal tar over a hundred year ago and used for saining of cells or micro-organisms when Paul Ehrlich actively worked. It has various unique biological activities and has been shown to be a useful fluorescent dye specific for DNA and RNA, a pH indicator, photosensitizer, anti-tumor and anti-malarial drug, and detector of bacteria and parasites. We recently found that AO accumulates in musculoskeletal sarcomas and that after illumination of the tumors with visible light or irradiation with low-dose X-rays, the dye rapidly exerts selective cytocidal effect against the sarcoma cells.
Methods: We have been applying surgery combined with photo- (PDT) or radiodynamic therapy (RDT) with AO (AO-PDT & RDT) to patients with musculoskeletal sarcomas after reduction surgery, to maintain an excellent limb function.
Results: The results of a clinical study on the outcome of this therapeutic strategy revealed that it yielded better local control and remarkably better limb function than wide resectional surgery.
Discussions: Based on our experimental studies, it was clarified that AO accumulates in acidic organelles or structures, especially lysosomes, depending on the acidity. An enormous number of protons are produced in cancer from lactate or CO_2 under hypoxic conditions, which are move into the extracellular fluid or lysosomes to maintain the intracellular fluid pH. Therefore, AO shows marked accumulation in the acidic lysosomes of cancer cells. Photon energy from visible light or X-rays excites the AO accumulated in lysosomes; the excited AO emits fluorescence and forms activated oxygen from intracytoplasmic oxygen. The activated oxygen destroys lysosomes, with the released lysosomal enzymes causing rapid death of the cancer cells. On the other hand, normal cells can exclude AO quickly because they are not acidic. Thus, AO-PDT and AO-RDT exhibit strong and selective cytocidal effect against malignant tumors.
Conclusions: We believe that AO-PDT and AO-RDT exhibit selective anti-cancer cell activity and that AO excited by photon energy has excellent potential as an anti-cancer agent like Magic Bullets which Paul Ehrlich suggested.

Extremely High Natriuretic Effect of 1-Desamino-8-homoarginine Vasotocin in Rats

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Background: Mammalian kidney retains sodium and therefore maintains extracellular fluid volume. Recently we have shown that vasotocin (VT) induces sharp increase in renal sodium excretion in rats (Gao, Natochin, 2004). It was important to synthesize new analogues of this hormone and to study their effect on renal functions in attempt to create a more effective and selective natriuretic peptide. In this work we studied a new VT analogue - 1-desamino-8-homoarginine vasotocin (1d-hAVT).
Methods: This study included 73 female Wistar rats: 43 rats were given the single intramuscular injection of 0.001 - 1 nmol/kg 1d-hAVT, 15 rats – 3 μmol/kg furosemide and 15 rats served as control. During the experiment the rats were kept in individual cages and urine samples were collected during 3 h. Urinary osmolality and electrolytes (Na, K) were measured by micro-osmometry and flame photometry, respectively. 1d-hAVT was synthesized by MI Titov, II Eliseev. ANOVA and Dunn post-hoc tests were used for statistical analysis.
Results: 1d-hAVT injection (1 or 0.5 nmol/kg) increased diuresis, sodium and potassium excretion (table), its action lasted approximately 2 h. Enhancement of natriuresis coincided with a high solute-free water reabsorption, which indicates preserved antidiuretic action of 1d-hAVT. It had the approximately 1.8-fold greater effect on sodium excretion than VT. 1d-hAVT and the loop diuretic furosemide have comparable natriuretic effect (p>0.05).

Treatment	Diuresis (μl per 2h)	$U_{Na}V$ (μmol per 2h)	U_KV (μmol per 2h)	T^c_{H2O} (μl per 2h)
1d-hAVT (1 nmol/kg)	1.14±0.23*	356±69**	123±34**	2.8±0.6**
Furosemide	3.98±0.71**	406±79**	103±12**	0.2±0.3
Control	0.24±0.17	10±8	23±17	0.5±0.3

Values are m±SD,* - p<0.05, ** - p<0.01 vs. control.

Conclusions: 1) The new VT analogue (1d-hAVT) has a high natriuretic effect and stimulates solute-free water reabsorption in the rat kidney. 2) Findings that 1d-hAVT at nanomolar doses induced natriuresis suggest existence of an intracellular cascade of signal enhancement through V-receptors. 3) Effectiveness of 1d-hAVT was 25000 times greater as compared with equimolar doses of furosemide. Using Ehrlich's term, 1d-hAVT act as the selective natriuretic "Magic Bullet".

Dynamics Based Design of Anti-Prion Compounds Uncovered the Hot Spots for Prion's Pathogenic Conversion Reaction

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Background: Prion proteins are key molecules in transmissible spongiform encephalopathies (TSEs). Although the precise mechanism of the conformational conversion process from the cellular form (PrP^C) to the scrapie form (PrP^{Sc}) is still unknown, we demonstrated that it is totally feasible to design a chemical chaperon which can stabilize the PrP^C conformation, and regulate the converison reaction. In oredr to design the chaperon, we utilized the slow dynamical information of a prion protein, and concomittantly identified the hots spots for pathogenic conversion reaction.
Methods: We conducted *in silico* screening to find compounds that fitted into a 'pocket' created by residues undergoing the conformational rearrangements between the native- and the sparsely populated high energy states (PrP*) elucidated by Carr-Purcell Meiboom-Gill relaxation dispersion method (NMR), and directly bind to those residues. Hit compounds were tested by *ex vivo* and *in vivo* screening, and if effective, they were subjected to determination of the complex structure and further lead optimization processes. The cyclic process between (1) structure determination, (2) *in silico* design, (3) organic synthesis, and (4) bioassay, termed Dynamics Based Drug Design (DBDD), was repeated recursively.
Results: More than hundred compounds were tested in a TSE-infected cell culture model, and more than twenty compounds including, 2-pyrrolidin-1-yl-N-[4-[4-(2-pyrrolidin-1-yl-acetylamino)-benzyl]-phenyl]-acetamide, termed GN8, efficiently reduced PrP^{Sc}. Subsequently, administration of GN8 was found to prolong the survival of TSE-infected mice. Heteronucler NMR and computer simulation showed that the specific binding sites are the A-S2 loop (N159) and the region from helix B (V189, T192 and K194) to B-C loop (E196), indicating that the intercalation of these distant regions termed 'Hot Spots' hampers the pathogenic conversion process.
Conclusions: Dynamics Based Drug Discovery (DBDD) strategy demonstrated here focusing on the hot spot of PrP^c will open the way to the development of novel anti-prion drugs.

Polymyxins: Differences & Similarities between Polymyxin B and Polymyxin E (Colistin), and their recent developments

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Hospital-acquired infections due to multidrug-resistant Gram-negative bacteria constitute major health problems, since the medical community is continuously running out of available effective antibiotics and no new agents are in the pipeline. Polymyxins, a group of antibacterials that was discovered during the late 1940s represent last treatment options for these infections. Commercially only two polymyxins are available, polymyxin E (colistin) and polymyxin B. Although several reviews have been recently published about colistin, no review has focused on the similarities and differences between polymyxin B and colistin. These two medications have many similarities with respect to mechanism of action, antimicrobial spectrum, clinical uses, and toxicity. However, they also differ in several points including chemical structure, formulation, potency, dosage, and pharmacokinetic properties. These differences will be described, with elucidation of their most recent developments

Oral Tolerance as a Method of Suppression of Immunological Response in Experimental Autoimmune Encephalomyelitis

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Background: Recently has been proposed to apply a method of oral tolerance to ameliorate auto-immune reactions. The aim of this study was to use the hydrolysate of pig spinal cord proteins (mixture of neuroantigens) to induce oral tolerance in the animal model of sclerosis multiplex (SM) - experimental allergic encephalomyelitis (EAE).
Methods: The female Lewis rats were fed with pig spinal cord hydrolysate in two doses for one week before immunization, which was induced by injection of guinea pig spinal cord homogenate. The clinical course was observed and evaluated in a five grade scale. At the peak of clinical symptoms (the 13th day post immunization) the rats were sacrificed and the spleen removed. Splenocytes were suspended in a culture medium and placed in microculture plates. The cells were stimulated with homogenate alone, hydrolysate alone, mixture of homogenate + hydrolysate, and medium alone. The cells were cultured for seven days. Subsequently, proliferation of splenocytes was estimated by means of {3H}thymidine incorporation and expressed in cpm (average of triplicate samples). In supernatants of cultures of splenocytes the level of cytokines interferon gamma (IFN-γ), interleukin (IL)-10, IL-4, and tumor growth factor (TGF)-α was measured.
Results: It was demonstrated that homogenate-induced splenocytes of hydrolysate-fed rats gave rise to low proliferation as compared to the controls used. The IFN-γ was inhibited in hydrolysate-fed animals as well as in hydrolysate-stimulated samples.
Conclusion: The results show that the hydrolysate of pig spinal cord proteins has a modulatory effect on the immune reaction, particularly on the orally-induced antigen-specific modulation of autoimmune response. It might have a clinical implication in SM treatment.

Interactions Between Drug Target Binding Sites and the Remarkable Story of Dopamine D1/D2 Synergism

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Background: Biological effects elicited by concomitant binding of two or more drugs to distinct but interacting proteins identifies these binding networks as potential drug targets. For example, recent findings on ligand-activated receptor dimerization and receptor–G protein–accessory protein coupling suggest a potentially useful approach based on Boolean logic: "AND" operations lead to fewer possible outcomes than "OR" operations. Translation of these concepts to biological processes gives rise to the concept of "Magic Bullet Cocktails." Twenty years ago, a remarkable discovery about signal transduction pathways in the brain was made, namely that the widespread effects of the neurotransmitter dopamine, with few exceptions, require concomitant agonist stimulation of *both* D1 *and* D2 receptor subtypes, a phenomenon referred to as "D1/D2 Synergism." Even more remarkable is the fact that shortly after depletion of synaptic dopamine, there is a breakdown in synergism: all of the effects of dopamine can be elicited by drug stimulation of *either* D1 *or* D2 receptors which are now profoundly supersensitive to stimulation.
Methods: We have used receptor autoradiography, behavioral analysis, differential display, and gene knockout techniques to identify the mechanism(s) of D1/D2 synergism and its breakdown.
Results: First, we showed that increases in dopamine receptor number cannot account for these changes. In order to identify unknown candidate genes whose expression may contribute to these phenomena, we used Differential Display of mRNA, which led to the discovery of a novel transcript later identified as *rhes*, a gene encoding a Ras homolog that functions as a G protein accessory protein. We found that conditions that lead to a breakdown in synergism and profound supersensitivity consistently result in decreased expression of *rhes* mRNA and Rhes protein. Furthermore, Rhes knockout mice are supersensitive to D2 but not D1 receptor agonists. Thus Rhes may normally serve to inhibit D2-metiated signaling.
Conclusions: A Magic Bullet Cocktail consisting of a drug that facilitates the action of Rhes in combination with a D1 and a D2 antagonist should provide a novel treatment for schizophrenia that is superior to existing therapies.

Melanoma initiating cells: new perspectives for therapy

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Keywords: melanoma, cancer stem cell, ABCG2, chemotherapy

Background: The model of cancer stem cells of tumor development states that tumor contain a subset of cells that both self renew and give rise to differentiated progeny. Recently our group identified a potential initiating/cancer stem cell subpopulation in melanoma which expresses ABCG2, an ABC-transporter involved in chemoresistance. Aims: 1) To identify and characterise melanoma CSCs, 2) To develop new therapeutic strategies for melanoma.
Methods: This study included human melanoma biopsies as well as human melanoma cell lines. In order to investigate the self-renewal capacity of these cells and the efficacy of new drugs, were carried out in vitro as well as in vivo studies.
Results: In human melanoma biopsy our group described a subpopulation expressing CD133 (Fig.1). Furthermore, a human melanoma cell line expressing high levels of CD133 was characterised and we described a subpopulation ABCG2 positive. CD133+/ABCG2+ cells were injected in NOD-SCID and, interestingly, the tumors expressed low levels of both markers accordingly to melanoma biopsies.

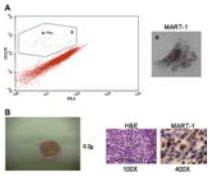

Fig. 1 Typical expression of CD133 in human melanoma biopsies (A) and tumour-initiating capability of CD133+ cells sorted and injected in NODSCID mice (B).
Conclusion: Considering that melanoma is one of the most aggressive forms of skin cancer and it is strongly resistant to conventional therapeutic agents, the molecular biology of CSCs opens interesting new perspectives from the pharmacological point of view.

The Many Lives of Hsp10: From Early Pregnancy Factor to Potential Antitumoral Agent. New Proteomic Data and a Review of the Literature Focusing on Its Immunologic Properties

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Background: Hsp10 expression has been investigated in several cancer models, with contrasting results. It is homologue to early pregnancy factor (EPF), a secreted protein which modulates the immune response of the mother versus the fetus. The impact of cigarette smoke (a major risk factor for lung diseases) on Hsp10 expression by airway cells has not been characterized yet.
Methods: We studied the effects of non-lethal doses of cigarette smoke extract (CSE) on the expression of Hsp60 and Hsp10 in human lung cells. Proteomics was carried out by 2D-IPG, silver stain, western blotting, and mass-spectrometry (MS). Database searches and chaperonomics were used to identify the proteins and genes of interest.
Results: Following CSE cell exposure as compared with unstressed cells, significant variations in Hsp10 did occur, in both lung fibroblasts and epithelial cells. In unstressed cells, three isoelectric variants of Hsp10 were found, which have not been reported for any other system, yet. After CSE exposure, only the most basic isoform was still expressed. To characterize the three variants found in unstressed cells, we performed MS analyses. Digested spots were analysed by nano-RP-HPLC-ESI-MS/MS to determine the fragments' amino acid sequences. Database searches showed that the most basic variant was human Hsp10 with 56% sequence coverage, and the other two isoforms had the same amino acid sequence, even if with a lower sequence coverage.
Conclusions: The data thus far indicate probably that Hsp10 protein variants are due to post-translational modifications. We recently showed the *in vivo* correlation between lung cancer development and downregulation of Hsp10 expression, and proposed a model for the antitumoral role for Hsp10, together with Hsp60. The precise role of Hsp10 in carcinogenesis is still unclear. The immunosuppressive activity of EPF/Hsp10 points towards a tumor-promoting role, mediating immune evasion and apoptosis resistance. On the other hand, the in vivo and in vitro evidences obtained in human lung models suggest that different Hsp10 isoforms may mediate diverse processes and should be differentially regulated.

Polypharmacy: A Major Risk In The Life Of The Elderly

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Addressing the high utilization of medications among the elderly, this study explored the degree of knowledge of *polypharmacy* and its consequences, as well as the pattern of utilization by a group of elders 65 years of age and over in the San Juan metropolitan area of Puerto Rico.
A dual technique was utilized: focus groups and a survey. Two focus groups were performed in order to explore issues pertaining the following domains: quality of life, doctor-patient and pharmacist-patient relationships, degree of knowledge of *polypharmacy,* and need of information. A questionnaire was administered to obtain information regarding their pattern of drug utilization.
Results of focus groups indicated that the elderly have learned how to cope with their chronic conditions and be able to continue with quality of life. Additionally, results suggested a need form the elderly to develop more assertiveness and closeness with their health care providers, both doctor and pharmacist; as well as a lack of sufficient knowledge of the serious implications that *polypharmacy* brings. Results from the survey revealed and confirmed their inappropriate utilization of medications, and suggest that this segment consults multiple physicians, all of which may bring potential problems of adverse drug interactions.
Further investigation is needed to examine fully the issue of *polypharmacy*, as well as the need of education that results in empowerment of patients, families and communities.

Biocompatible Nanoparticles, Carriers Of The Magic Bullet

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Background: Current barriers to cancer chemotherapy include i) toxic side effects, ii) the limited accessibility of the drugs to tumor tissue, and iii) multi-drug resistance developed by malignant tumors during treatment. We developed a novel drug delivery system utilizing reconstituted high density lipoprotein (rHDL) nanoparticles with reduced toxicity to normal tissues and selective receptor-mediated uptake of anti-cancer drugs**.** The rHDL formulation is preferred over conventional drug delivery strategies because its small size and biocompatible components. A further advantage of the rHDL drug delivery model is the selective lipid uptake mechanism by which drugs are delivered to target cells via the scavenger receptor, class B, Type I (SR-BI).
Methods: The rHDL/paclitaxel (PTX) nanoparticles were characterized with regard to size, shape, stability and cytotoxicity against cancer cells using the MTT assay. Maximum tolerated dose studies were performed in C57Bl/6 female mice comparing rHDL/PTX with Taxol® and Abraxane®. In tumor suppression studies, the .
Results: The rHDL/PTX nanoparticles were found to have a diameter of 11.4 +/- 3.1 nm and 5-20 fold enhanced toxicity against cancer cells when compared to free PTX. the majority (82%) of the paclitaxel was taken up by cancer cells via a selectivel uptake mechanism, apparently via the SR-B1 receptor. Incubation of the cells with HDL_3, the natural ligand of SR-BI, supressed paclitaxel uptake to 30.6% as compared to rHDL/Ptx alone ($p<0.0001$) supporting the specificity of the receptor uptake mechanism. During studies with mice a 2.3-fold and 1.4-fold higher dosage of rHDL/Ptx could were tolerated by mice, compared to Taxol® and Abraxane®, respectively. Recent tumor suppression studies with mice show that the rHDL delivery system is highly effective in reducing the tumor burden in mice carrying xenografts of human tumors.
Conclusions: Reconstituted high density lipoprotein (rHDL) provides a targeted delivery vehicle for the encapsulate paclitaxel via receptor mediated uptake of the drug by cancer cells and tumors. Encapsulation of chemotherapy drugs in rHDL enhances the tolerance of the drug while increases ist toxicity against cancer cells and tumors. The rHDL nanoparticles should thus reduce the toxic side effects seen with other formulations while enhancing the anti-tumor effectiveness of the encapsulated drug

Blood Choline Phospholipids As Preferential Sources Of Docosahexaenoic Acid To The Brain

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Docosahexaenoic acid (DHA) is the main polyunsaturated fatty acid (PUFA) of the brain phospholipids. It plays a major role in the brain development, learning activities and visual acuity. As a highly unsaturated molecule, it is supposed to be very sensitive to peroxidation, and it has been reported to be degraded, presumably in response to oxidative stress, in number of neurodegenerative diseases.
The accretion of DHA to the brain has been assumed to be done from the blood circulating pool associated to albumin, DHA being in its unesterified form which can cross the blood-brain barrier (BBB). Blood albumin also carries lysophospholipids, mainly lysophosphatidylcholine (LysoPC). We first found that the uptake of DHA by the brain was aound ten-fold more efficient when DHA was esterified in LysoPC compared with unesterified DHA (Thiès et al 1994). Then we found that DHA ingested either in triglycerides (oil) or phosphatidylcholines (PC) may circulate as DHA-containing LysoPC (LysoPC-DHA) in a way which is compatible with an efficient uptake of DHA under this form (Brossard et al 1996, 1997 & Lemaitre-Delaunay et al 1999). Furthermore, an *in vitro* reconstituted BBB allowed to find a preferential crossing of LysoPC-DHA over DHA (Bernoud-Hubac et al 1999). Also, a substantial amount of DHA has been found to circulate in the form of LysoPC-DHA with DHA at the *sn*-2 position (the usual position of PUFA in phospholipids), although rapidly isomerizing into *sn*-1-LysoPC-DHA (Croset et al 2000).
A recent work from Chen and Subbaiah (2007) has brought evidence for a preferential cleavage of DHA-containing PC by the so-called endothelial lipase which releases *sn*-2-Lyso-PC-DHA, making relevant this form of LysoPC-DHA for an efficient uptake by the brain.
As *sn*-2-Lyso-PC-DHA is rapidly isomerized into its *sn*-1 position isomer, we have set up a one step method (patent 2008) to produce 1-Acetyl,2-DHA-PC (AceDoPC) of which the structure is closely related to LysoPC-DHA. This method will be used to prepare ^{13}C-labeled DHA-containing choline phospholipids in order to perform new human studies on the metabolic fate of DHA when ingested in those different forms. This work is in progress.
It is concluded that LysoPC-DHA or its stabilized form AceDoPC may be efficient carriers of DHA to the brain, and then could be used as a way to compensate for DHA-depleted brains, whatever the reason of such a depletion.

Desining Novel Antiinfective Concepts Combining Nanotechnology, Bioplastics and Natural Products

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Abstract: This presentation reviews a number of research efforts carried out within our group where combining natural products, nanotechnology and biopolymers can be of value in the pharmaceutical and biomedical areas to design novel efficient antiinfective systems. The talk presents first our recent efforts to understand and optimize the antimicrobial properties of chitosan and electrospun nanofibers of chitosan and of other antimicrobial biomass derived biopolymers and blends (see Figure 1). It does later describe our most recent efforts to design antiinfective and bioactive bone replacement interphases and wound dressing systems based on nanostructured fiber mats of biopolymers carrying biocides and carried out within the EU FP6 project NEWBONE. Finally, the presentation describes the capacity of certain nanoclays to intercalate and control release biocide and bioactive plant extracts (see Figure 2) and, within a very recent collaboration with the University of British Columbia and Risoe DTU, of pharmaceutical antibiotics such as tetracycline.

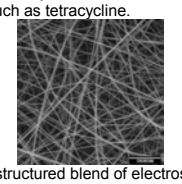

Figure 1. Antiinfective nanostructured blend of electrospun PLA-chitosan

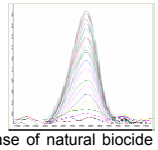

Figure 2. Following the release of natural biocide thymol by FTIR spectroscopy from resorbable clay nanobiocomposites of PCL

Acknowledgements: The authors would like to acknowledge the EU FP6 project SUSTAINPACK, the CSIC overseas support program (A12008PM1), the Spanish research project MAT2006-10261-C03 and Nanobiomatters Ltd. for financial support.

Selection Of Cell Culture Substrate For Human Viral Vaccines

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Background: Many cell cultures for human viral vaccines used up today, animal origin or human diploide cells (Wi-38), primary culture or continued cell lines like VERO. The rabies vaccine produced on BHK-21/C-13 (Baby Hamster Kidney) cell culture has been used for a long time in animals. Since the safety of the BHK cells for animals is not questionable, the possibility of their use for a human rabies vaccine has been discussed. Contamination by cellular DNA is not dangerous becouse the treatment with beta - propiolactone during virus inactivation completely inactivates the biological activity of DNA as well. This finding, which was accepted also by WHO, encourages the use of BHK cells as substrate for human vaccine production.
Methods: We multiplied L. Pasteur strain of rabies virus on the BHK-21 (C-13) cells and produced beta-propiolactone inactivated and aluminium-phosphate adsorbed rabies vaccine. A total of 300 adult subjects were vaccinated. A clinical testing was conducted with three doses of vaccine, intramuscularly in the deltoid region by the pre-exposition scheme 0-7-21 days. At 30th day post vaccination serum antibodies were measured by the RRFFIT (Rapid Rabies Focus Fluorescence Inhibition Test).
Results: In comparation with VERO cell line of monkey origin, we harvested one log more rabies virus from BHK cells and vaccine production is possible without virus concentration. Local reactions in few percents and no sistemic adverse reactions were registered. All vaccinees had antibody titer over acceptable mimimal (0,5 UI). We also adapted polio and measles viruses on BHK cells for possible vaccine production.
Conclusions: We conclude that this rabies vaccine is low cost, safe and effective for humans. The preliminary results with BHK/21 vaccine in volunteers confirmed its good tolerability and immunogenicity. This product designed is safe on the basis of experimental results that virus inactivation by beta-propiolactone destroyes contaminant DNA from cell culture.

Magic Bullets: Beynod Selective Targeting to Selective Killing Using Armed Antibodies

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Background: In recent years, there has been increasing interest in the use of highly toxic small molecules as the attached cell-killing agent. One reason for the heightened interest in the field is the increase in the number of companies pursuing antibody-based anticancer agents, since many tumor-targeting antibodies identified as a result of this effort lack meaningful anticancer activity of their own. Creating antibody-maytansinoid conjugate (AMC) compounds provides a means of achieving effective products from such antibodies.
Clinical Validation of Maytansinoid Technology: Currently eight AMC compounds are in clinical testing using ImmunoGen's maytansinoid technology, with one more expected to enter the clinic in 2008. One of the compounds in the clinic, T-DM1, is a conjugate of the maytansinoid DM1 with the antibody trastuzumab. It is being evaluated in patients with HER2-expressing metastatic breast cancer that have progressed on treatment with a chemotherapy regimen that includes trastuzumab. The initial clinical data reported is encouraging, and includes a confirmed objective response rate of 44% (4/9) at a dose of 3.6 mg/kg q3weeks in a Phase I trial in such trastuzumab-nonresponsive patients.
Understanding the Mechanism of Cell Killing by AMCs: The maytansinoid molecules are linked to the antibody molecule at lysine residues via a disulfide or a non-reducible thioether link. The disulfide linkers are designed with steric hindrance at carbon atoms adjacent to the disulfide bond to maximize plasma stability of the conjugate and to facilitate the intracellular release of the maytansinoid by disulfide-reduction. The non-cleavable thioether link is designed to be stable in plasma, and the conjugate upon binding and internalization in the target cancer cell is lysosomally processed to release the thioether-linked maytansinoid attached to the lysine residue.
Broadening the Technology: Evaluation of the role of linkers in the effective intracellular release of the cytotoxic maytansinoid metabolites has led to the creation of novel hydrophilic linkers that are stable in plasma and yield even greater efficacy to the conjugates based on *in vitro* and *in vivo* pre-clinical studies. The hydrophilic linkers confer improved activity against multi-drug resistant (mdr) cancer cells, and also offer potential for agents against tumors that express the target antigen at low density.

Aerosolized Liposomal Antifungal Agents

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Background: In the past decade three classes of antifungal agents, polyenes, azoles, and echinocandins have been extensively studied and used. One of the most important antifungal agents, Amphotericin B (AmB), a polyene, has toxicity that limits the lung tissue doses that can be achieved through intravenous administration. Incorporation of AmB in liposomes reduces the toxicity and increases the therapeutic index for intravenous administration. Targeted delivery to lung tissues (those usually first colonized and infested by fungi) via inhaled liposomal AmB aerosol is an effective approach. Development of optimal aerosolized liposomal AmB therapies requires a better understanding of the effect that liposome surface charge has on lung clearance kinetics. In this work we evaluated the clearance kinetics and organ distribution of inhaled liposomal AmB in male Balb/C mice.
Methods: Mice were exposed via nose only to AmB-containing liposomal aerosols having positive, negative, or neutral surface charge characteristics. The formulations were aerosolized using a Collison nebulizer. Groups of animals were euthanized at predetermined times. The lungs and other organs were analyzed for AmB using an HPLC method. AmB was not detected in serum or other organs such as kidneys, liver, and brain.
Results: The disposition of neutral and positive liposomal amphotericin B in lungs followed biexponential kinetics. The alpha and beta phase half-lives for positive liposomes were 1.3 and 15.1 days, respectively, and 2.3 and 22 days for neutral liposomes. AmB delivered via negative liposomes exhibited monoexponential clearance with a half-life of 4.5 days.
Conclusions: These results suggest that toxic side effects in nontarget tissues are minimal and may indicate a potential for long term protection against fungal infections.

Magic Bullets And Vaccines: Learning From The Brain

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Background: For developing magic bullets and vaccines, the brain is of interest in two different ways: 1) The brain is an important target organ in its own right. 2) The brain's low background level of immune activity allows general principles to be revealed.
Methods: Evolving understanding about delivery of magic bullets and about therapeutic vaccines is reviewed. Examples are taken from rat models that include: local cytokine injection, autoimmune inflammation, implanted tumor, and blood-borne metastases (1-4).
Results: "IMMUNE PRIVILEGE." 1) Immune activity is regulated in the brain – just as in other organs, and the normal baseline is low. However, the brain is not "privileged." Both beneficial and harmful immune responses can occur. 2) The low baseline affects development of vaccines that target the brain. Most effort has gone to identifying appropriate antigens and initiating a response. It is just as important to enhance the effector phase within the brain. 3) The low baseline makes it possible to see subtle effects that are relevant to all organs. In the brain, as in other organs, individual regulatory molecules have many functions and affect many cell types. The neuropeptide, substance P, and the neurotransmitter, glutamate, also affect immune regulation. BLOOD-BRAIN BARRIER (BBB): its role is often misunderstood. 1) The normal BBB does indeed prevent the passive entry of large proteins and many drugs. 2) The BBB is not normal at tumor masses or other sites of pathology. However, other factors can also impede drug entry. 3) The BBB does not prevent the entry of metabolically-active, migratory cells. However, other factors are important, if cell-mediated responses are to be manipulated. **Conclusions:** Misconceptions have hindered research in the brain. Increased understanding can aid delivery of new drugs and improve success with vaccines. The brain's low baseline allows subtle effects, such as the role of local regulatory molecules, to be revealed.

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Clinical Guidelines For The Use Of Extended Interval Dosage Regimens Of Gentamicin In Neonates

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Background: Development and validation of guidelines for gentamicin dosing in neonates using extended-interval dosage regimens.
Methods: With the base of previously obtained population pharmacokinetic parameters (JM Lanao. JAC 48:1038-48,2004), dosing guidelines were designed to achieve serum gentamicin concentrations (SGCs) within the ranges considered therapeutic in adults for extended-interval dosing (peak 15-20 mg/L and trough <0.5 mg/L). These guidelines were adopted as the dosing practice at our Institution.
The validation population comprised 81 neonates dosed according to the proposed guidelines, routinely monitored, with the following clinical characteristics: gestational age (GA) 24 - 40 weeks (mean (SD); 33.48 (4.42)), and postnatal ages of 1 - 11 days (2.80 (1.52)). C-reactive protein (CRP) and serum creatinine were measured at the start and end of treatment for the evaluation of efficacy and toxicity of the treatment.
Results: In term newborns and premature babies with GA between 31 – 38 weeks, extended-interval dosage regimens with initial gentamicin doses of 10-12 mg/kg and dosage intervals of 36-48 h are recommended. Owing to their high distribution volumes and prolonged half-lives, for premature babies of GA <31 weeks we recommend initial doses of 5 mg/kg and dosage intervals of 36-48 h to reach SGCs between 0.5-10 mg/L.
A linear relationship between the individualized dose after SGCs monitoring (ID) and guideline dose (GD) was obtained: ID = 0,9469 GD - 0,909; r^2 = 0,8991. A statistically significant difference ($p<0.05$) was found between initial and final CRP levels in patients with sepsis (2.45 (1.38) vs 1.29 (1.56)mg/dl) or suspected infections (2.04(1.85) vs 0.87 (0.56) mg/dl). A statistically significant decrease in the serum creatinine concentration was also observed ($p<0.01$).
Conclusions: The pharmacokinetic and clinical validation of the guidelines developed suggests that they are efficient and safe for the initial dosing of gentaminicin in term and premature babies.

Erythropoietin in Cancer Anaemia: Friend or Foe?

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Erythropoietin (EPO), a glycoprotein hormone produced mainly in the kidney and the liver, binds to the EPO receptor (EPO-R) on erythroid precursor cells in the bone marrow, thereby promoting their survival, proliferation, and differentiation. In adults normal erythropoiesis produces about 2.3 million red blood cells per second, regulated by basal levels of 0.8–4.0 pmoles/L of EPO (5–25 U/L) in plasma.
Many cancer patients suffer from anaemia, and recombinant human EPO and other erythropoiesis stimulating agents (ESAs) are widely used therapeutically, to increase haematocrit, lower blood transfusion requirements, and improve quality of life. However in recent years, several investigators have identified EPO-R expression in numerous cancers and tumour cell lines, raising concerns about the safety of ESA therapy for cancer-associated anaemia. Pharmacological doses of EPO elevate plasma concentrations several fold and potentially could modulate tumour growth.
In 2006, a Cochrane Review collated data on over 9000 cancer patients from 57 trials in which recombinant EPO or darbepoetin alfa was given to prevent or treat anaemia. ESA-treated patients had significantly lower blood transfusion requirements. Although there was no significant difference in survival between ESA- and placebo-treated patients, none of the trials included in the meta-analysis had sufficient statistical power to confidently determine the effects of ESAs on overall survival. The relative risk for thromboembolic events was much higher in ESA-treated patients compared with controls [1, 2]. Overall, these studies have raised concerns that ESAs could, in certain circumstances, adversely affect survival in cancer patients. It has been speculated that these agents may enhance thrombosis, tumour growth, and neovascularization. In 2007, the FDA issued safety warnings alerting clinicians to the potential harm associated with ESA therapy for cancer-associated anaemia.
Continued scrutiny of clinical trials, particularly survival data, and current clinical practice patterns is important to fully understand the risk–benefit ratio of ESA treatment in anaemic cancer patients.

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From Classic Autohemotherapy To Autologous Hemoderivative Cancer Vaccine Through A Drug And Drug-Carrier Immunomodulatory Adjuvant System

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Background: From 1898 to 1940, limited but repeated successful results of autohemotherapy (AH) in several diseases including cancer were described. The interest on AH dimmed after the appearance of antibiotics and chemotherapy. Discovery of serum tumor markers confirmed a cancer AH paradigm, the release of potential antigens from tumor to the blood. Revisiting AH allowed to demonstrate and to potentiate AH immunomodulatory activity. Since 1995, 20 peer review publications reported the development of an Autologous Thermostable Hemoderivative Cancer Vaccine (ATH-CV). In this unpublished study, ATH-CV was used as immunomodulatory pretreatment of chemo in advanced Non Small Cell Lung Cancer (NSCLC), hypothesizing a new strategy with ATH-CV as anti-tolerogenic adjuvant and chemo as endogen vaccination.
Methods: *Patients* (60) in randomized Phase II 2-arm trial (A1: Chemo, A2: Chemo after ATH-CV). *Eligibility*: NSCLC, stage 4, no previous chemo, PS ≤ 2, available tumor sample to prepare tumor lysate (TL) for immunity tests. *Exclusion*: Brain metastasis, immunity disease. *Assessments*: Delayed Type Hypersensitivity elicited by TL (DTH-TL); IFN-γ Elyspot challenged with TL (ELY-TL); immunophenotyping of lymph node cells (IPh-LNC) and peripheral blood mononuclear cells (IPh-PBMC) measuring activated dendritic cells (aDC) as CD1a+CD83+ and T-Regulatory cells (T-Reg) as CD4+CD25+FOXP3+; 30-day Tumor Growth (30-TG); survival curves (Kaplan & Meier); Median Survival Time (MST); 1 Year Overall Survival (1-OS).
Statistics: A1, A2 assessments were compared by Student-t and Log-rank tests.
Results:
At pre-chemotherapy
IPh-LNC (% A1, mean ± SD) aDC: A1=100 ± 12, A2=380 ± 16; T-Reg: A1=100 ± 8, A2=32 ± 5.
IPh-PBMC (% A1, mean ± SD) T-Reg: A1=100 ± 14, A2=24 ± 4.
DTH-TL (% cases+) **A1**=0%, A2=30%.
ELY-TL (spots by 10^6 target) A1=24 ± 1.1, A2=108 ± 6.2.
30-TG (%) A1=34 ± 12, A2=16 ± 6.
At trial day 120
DTH-TL A1=5%, A2=60%.
ELY-TL A1=54 ± 12, A2=325 ± 36.
30-TG A1=21 ± 4, A2=12 ± 3.
At trial day 360
MST (wks) A1=28, A2=38.
1-OS (%) A1=30, A2=42.
Conclusions: 1) ATH-CV switched IPh-LNC increasing aDC and depleting T-Reg ($p<0.02$); 2) ATH-CV pre-treatment, increased anti-tumor Chemo effect ($p<0.01$); 3) Chemo after ATH-CV enhanced anti-tumor immune response ($p<0.02$).

Temperature, Denaturant And Ligand Effects On Solution Stability And Conformational Properties Of Human Interleukin-1 Receptor Antagonist

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Background: A thorough understanding of solution stability and aggregation of protein therapeutics is important for successful manufacturing and delivery of protein drugs. Aim: To investigate stability and structural properties of a therapeutic anti-inflammatory cytokine, human interleukin-1 receptor antagonist (IL-1ra).
Methods: HPLC, optical spectroscopy, high resolution 2D NMR and small angle X-ray scattering (SAXS).
Results: The results are consistent with a variety of non-cooperative changes within the folded state ensemble of the protein. In particular, the interface between the first and the second folding motifs was progressively destabilized by increasing urea concentrations. This region outlines an intrinsically labile part of the folded structure which undergoes perturbations with no detectable exposure of hydrophobic surfaces. Additional evidence for non-cooperative unfolding of IL-1ra comes from nonuniform peak intensity variations, weighted average chemical shift differences, as well as bell-shaped denaturation profiles for some of the minor peaks.
In addition, binding of 8-anilinonaphthalene-1-sulfonic acid (ANS) to IL-1ra was investigated to address protein aggregation in the presence of this low molecular weight compound. Effects of ambient to elevated temperatures on the affinity and specificity of ANS binding were assessed. Overall, the affinity of ANS was lower at 37 °C compared to 25 °C, but no significant change in the site-specificity of binding was observed from the chemical shift perturbation data. No evidence was found for any partially denatured or aggregated forms of IL-1ra throughout the experimental conditions, consistent with a cooperative and reversible denaturation process. The results support earlier observations on the tendency of ANS to interact with solvent exposed positively charged sites on proteins.
Conclusions: 1) Equilibrium unfolding of IL-1ra is associated with accumulation of highly native-like intermediates rather than molten globule-like states. 2) ANS binding occurs within a previously identified aggregation-critical region in the vicinity of the intrinsically labile part of the structure, thus providing an insight into the ligand-dependent aggregation of IL-1ra.

Innovation in Anticoagulation: Discovery and Development of Novel Small-Molecule Coagulation Inhibitors as New Treatment Options for Thromboembolic Diseases

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Background: Thrombosis, defined as the formation or presence of a clot in a blood vessel leading to ischaemia, infarction, or organ damage, is a leading cause of death and disability in the Western world. Prevention or treatment is presently managed by heparins or vitamin K antagonists (VKAs), although clear drawbacks exist with these treatments, especially in regard to long-term use. Such drawbacks include parenteral administration, unpredictable pharmacology, extensive drug or food interactions, a narrow therapeutic window, and a need for monitoring. However, the discovery of oral, direct inhibitors of thrombin and Factor Xa (FXa) showed that it might be possible to overcome these limitations. In particular, inhibition of FXa is an attractive target for novel, orally available anticoagulants, because FXa occupies a central position in the coagulation cascade and, unlike thrombin, has no other known functions.
Methods and Results: Rivaroxaban is an oral, direct FXa inhibitor that has received a positive CHMP recommendation for the prevention of venous thromboembolism (VTE) after elective total hip or total knee replacement surgery (THR/TKR), and is also approved in Canada for this indication. It is also in advanced clinical development for the prevention and treatment of other thromboembolic disorders. Rivaroxaban is a highly selective inhibitor of FXa (Ki of 0.4±0.02 nM) and its antithrombotic effect has been demonstrated in arterial and venous thrombosis animal models. At effective antithrombotic doses, rivaroxaban does not significantly prolong bleeding times, in contrast to the VKAs. In phase II studies, rivaroxaban was effective and well tolerated in the prevention of VTE after THR or TKR, as well as in the treatment of deep vein thrombosis. In phase III studies in TKR and THR (RECORD1–4), various rivaroxaban regimens demonstrated significantly superior efficacy to enoxaparin regimens for thromboprophylaxis, with similar rates of major bleeding. Rivaroxaban is also being assessed for the treatment and secondary prevention of VTE, stroke prevention in atrial fibrillation and secondary prevention in acute coronary syndrome.
Conclusion: Rivaroxaban offers the potential to overcome the limitations of current pharmacological agents in the prevention and treatment of thromboembolic disorders.

Vitamin C In Intravenous Nutritive Solution: Double-Edge Effect For Premature Newborn Infants

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Background: Premature infants are at risk of pathological complications related to oxidative phenomenon. Among them, the incidence of broncho-pulmonary dysplasia (BPD) is near of 40% in newborns of < 28 weeks of gestation. Because of the immaturity of their gastro-intestinal system, these children are frequently nourished by intravenous way. Although this parenteral nutrition (PN) contains antioxidant vitamins such as vitamin C, this solution is contaminated by peroxides. This contamination is associated with inadequate proto-protection of nutritive solution. The interaction, catalyzed by photo-exited riboflavin, between vitamin C and oxygen dissolved in solution generates hydrogen peroxide (H_2O_2). The infusion of PN without photo-protection to newborn guinea pigs induces a lower amount of alveoli in lungs, a characteristic feature of BPD. This observation is not induced by an infusion of H_2O_2, vitamin C or riboflavin alone, but with the combination of vitamin C + riboflavin. The interaction between dehydroascorbate and H_2O_2 generated in PN leads to the formation of a new compound, named ascorbylperoxide (2,3-diketo-4-hydoxyperoxyl-5,6-dihydroxyhexanoic acid). We hypothesis that ascorbylperoxide is the active agent leading to a low alveoli development in newborns.
Methods: Three days old guinea pig pups received intravenous solution containing increasing concentrations of ascorbylperoxyde. After 4 days, lungs were samples for histological determination of alveoli. Ascorbylperoxide concentrations in PN as well as in urine samples were determined by mass spectrometry.
Results: The alveoli count was negatively correlated (r^2 = 0.64; p<0.01) with urinary logarithmic concentration of ascorbylperoxide. The addition of glutathione into PN solution allows the recycling of DHA in ascorbate, thus preventing the generation of ascorbylperoxide and degradation of vitamin C.
Conclusion: Results suggest that the interaction of ascorbate with other components present into PN such as riboflavin and oxygen contributes greatly to development of BPD in premature infants. The addition of glutathione to PN could prevent the loss of alveoli and improve the availability of vitamin C.
Supported by Canadian Institutes of Health Research (MOP 79403)

Controlled Release Amoxicillin Therapy In Veterinary Medicine

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Antibiotics are the most commonly prescribed drugs in small animal medicine that are applied in a variety of microbial infections. The most common veterinary treatment protocol is performed on 'outpatient' basis and involves oral treatment with beta-lactam antibiotics, such as ampicillin, amoxicillin, amoxicillin-clavulanic acid, cephalexin and cefuroxime. The short biological half-life of beta-lactam antibiotics and their pharmacodynamic properties that require prolonged exposure of the pathogen to the effective drug concentrations necessitate multiple daily dosing of the beta-lactam drugs throughout the treatment period that usually lasts for 5-7 days.
The major drawback of antimicrobial treatment protocols is related to the fact that effective antimicrobial therapy requires multiple daily drug administrations during the treatment period. As a result, one of the common reasons for failure of antimicrobial therapy is the low drug compliance by the pet owners. The prime motives for this poor compliance are: being out of the house during the day, difficulties with restraining the animal, and lack of confidence.
We are developing an effective solution to the low drug compliance problem by development of oral dosage form for beta-lactam antibiotics that after a single administration would provide effective drug concentrations throughout the whole treatment period.
The main drawback in the design of single oral administration treatment strategies for beta-lactam antibiotics is the fact that these drugs are absorbed only in the small intestine and thus have a narrow 'absorption window' with no colonic absorption and the drug effect terminates shortly after the formulation reaches the colon. The approach of a single dose controlled release antibiotic therapy (SCRAT) is based on expandable swelling matrix tablet with prolonged retentivity in the stomach that releases the drug over several days. Thereby, enables continuous input of the beta-lactam drugs to the "absorption window" at the upper parts of the gastrointestinal and ensure treatment of the infection over several days following single administration. Thus, SCRAT provides a means to utilize the major pharmacokinetic and pharmacodynamic advantages of controlled release dosage forms for beta-lactam antibiotics.

Folate-Targeted Chemotherapy

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Background: The small molecular weight ligand, folic acid, is capable of targeting covalently attached bioactive agents quite specifically to folate receptor (FR)-positive cancers. To date, impressive anti-tumor activity has been observed preclinically with folate conjugates of potent chemotherapeutic molecules, and a few of these agents have recently entered the clinic with more to soon follow. In this present investigation, we explored the possibility of using the folate ligand to target a potent, semi-synthetic analog of the microtubule inhibitor, tubulysin B, to FR-enriched tumors.
Methods: EC0305, a folate-tubulysin conjugate, was evaluated *in vitro* for dose-dependent cytotoxic activity against a panel of FR-positive and negative cells. Cells were pulsed with EC0305 for 2 h in the presence and absence of excess folate, and then chased in fresh medium up to 72 h. *nu/nu* mice (Balb/c background) were inoculated with FR-positive KB cells, and 74 ± 14 mm^3 tumors were established 11 days later. EC0305 was then administered through the lateral tail vein, and tumors were measured every 2-3 days using a caliper. Tumor volumes were calculated and then compared to untreated controls.
Results: EC0305 was found to specifically inhibit the growth of a panel of FR-positive cell lines (IC_{50} range 1 to 10 nM) in a dose-dependent manner, whereas cells lacking FR expression were unaffected. EC0305's potency was also confirmed against a human KB xenograft-nu/nu mouse cancer model. Here, a brief three times per week, 2 week regimen yielded remarkable anti-tumor activity (100% tumor-free animals) without causing significant weight loss or major organ tissue degeneration. In contrast, anti-tumor activity was completely abolished in EC0305-treated animals that were co-dosed with an excess of a nontoxic folate-containing analog, thereby confirming that this agent's antitumor effect was mediated by FRs. The advantage provided by folate conjugation was further proven by the un-targeted free drug, which was found to be completely inactive at both tolerable and highly toxic dose levels.
Conclusions: These results collectively show that a potent anti-proliferative tubulysin compound can be specifically delivered to FR-positive tumors to provide substantial therapeutic benefit using well-tolerable dosing regimens.

Hydrophilic hexapeptides – a new class of ATF – dependent transport proteins of multiple drug resistance

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Multiple drug resistance (MDR) appears as a result of shart rise in pumping out medical products from a cell into extracellular space by ATF – dependent transport proteins. At present no transport proteins effective inhibitor is available that could be used for the MDR overcoming. The effect of three hydrophilic hexapeptides, including cyclic hexapeptide on activity and amount of transport proteins responsible for MDR formation has been investigated. Cell lines of human throat cancer, Hep2, human oral cavity carcinoma, KB 8-5, and human prostate cancer, PC – 3, have been used. The examination of specified hexapeptides effects was performed. The examination of specific hexapeptides effects was performed by comparison of Rh 123 output intensivity and transport proteins and their genes expression values. It has been shown that hydrophilic hexapeptides inhibit substrate output from a cell into extracellular space in concentrations of 10^{-10} M.
However, the effect of Hydrophilic hexapeptides on the MDR modulation is carried out by different mechanisms of action. It was unknown so far the tumor multidrug resistance inhibitors, which showed activity in such low concentration.

Effects of Central Penicillin Administration on Neuronal Response of the Nucleus Reticularis Gigantocellularis

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Background: Reticular neurons of medulla oblongata participate in a large variety of sensory, motor and autonomic functions. The gigantocellular reticular nucleus is a part of brainstem neuronal network with a high density of GABAergic neurons. This structure plays the regulatory, integrative and coordinative role in cardiovascular and respiratory control. The aim of this paper was to study the neuronal responses of bulbar reticular units after disturbance of inhibitory processes.
Methods: Penicillin was used as a convulsant agent, which is able to non-selectively block $GABA_A$-mediated synaptic inhibition. Benzyl penicillin (50 U/μl) was locally applied by pressure microinjection into the central part of reticular gigantocellular nucleus of 65 anesthetized or paralyzed Wistar rat. Effects of penicillin administration were examined on the firing rate, discharge pattern and interspike intervals of reticular units.
Results: Penicillin caused enhancement of the number of active firing neurons (138%) and reorganization the spatial neuronal architecture of the examined structure. In gigantocellular nucleus penicillin application induced excitatory responses in 78% of extracellularly recorded units and inhibition in 15% of them. 18% of recorded neurons exhibited high-frequency firing activity. These facts support data which showed that this reticular region includes functionally labile cells capable of transforming tonic activity into burst pattern. Penicillin microinjection increased the mean firing rate of reticular neurons from 9.63±1.24Hz to 12.11±1.59Hz ($p<0.05$).The peak and mode of interspike intervals histograms were shifted toward shorter intervals and their amplitude was increased after penicillin microinjection. Distributions of interspike intervals histograms were transformed (unimodal to exponential, multimodal to unimodal). In no case did vehicle administration produce the significant changes in neuronal activity.
Conclusion: We concluded that neurons of reticular gigantocellular nucleus are very sensitive to the local blockage of synaptic inhibition by penicillin within of medulla oblongata. Results suggest a considerable physiological role of medullary inhibitory mechanisms in homeostasis maintenance.

The Role Of Central Vagal Control In The Action Of Lipophilic And Hydrophilic Beta-Adrenoblockers

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Background: The effects of systemic administration of beta-adrenoblockers on the chronotropic heart functions are well known, however little is known about its influences to the heart rate variability and central effects of these drugs. In the present study we aimed to compare the efficacy of lipophilic beta-adrenobloker Propranolol to that of hydrophilic beta-adrenoblocker Sotalol by various methods of administrations.
Methods: Experiments were performed in anesthetized and artificially ventilated 51 white outbred rats. The heart rhythm disturbances caused by occlusion of the left coronary artery. ECG was recorded in three standard leads. To reveal the central effects of the drugs intracisternal injections were made (Propranolol at dose to 10 μg per animal, Sotalol - to 250 μg) throw the occipital membrane, to assess the peripheral effects the drugs were administrated intraperitoneally prior coronary occlusion (Propranolol at dose 1mg/kg, Sotalol - 5 mg/kg).
Results: In the control experiments coronary occlusion leaded to polymorphous cardiac arrhythmias and ventricular fibrillation was developed. Propranolol produced the reduction of all cardiac disturbances observed after coronary occlusion irrespective of the methods of administrations. Its intracisternal injections led to increase of RR interval (161.35±3.4 ms to 181.5±4.6 ms, $p<0.01$), Sotalol had effect only by introperitoneally injections. Propranolol caused the enhancement of total spectral power of the heart rate variability and all spectral components. The pronounced effect Propranolol of the HF component was found. The bulbar brain stem structures participate in regulation of cardiac functions. The HF component may reflect mainly the fluctuating activity of vagal center. Thus Propranolol contrary to Sotalol manifests the central effect possibly to its lipophylic properties.
Conclusion: Results allow concluding that more pronounced activity of Propranolol is probably associated with its lipophylic profile, and it is also likely to depend on Propranolol-induced activation of the central vagal path from medulla oblongata to the heart.

Fentanyl: How Delivery System Can Modify Clinical Properties Of Molecule

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Fentanyl (N-phenyl-N-(1-phenethyl-4-piperidinyl)propanamide) was first synthesized by Paul Janssen in 1959. Fentanyl was introduced into medical practice in the 1960s. It has long been established in anesthetics and ITU practice due to potent analgesic action, intravenous administration, very rapid onset, short half-life and low incidence of histamine.
Fentanyl administrated orally has low bioavability due to first pass metabolism. Intravenous route of administration have limited significantly fentanyl application outside anesthesia and ITU until “magic bullets” was applied. Fentanyl “magic bullets” have form of different delivery systems, which significantly modify clinical properties of molecule.
Development of transdermal fentanyl patches allows avoiding intravenous route of administration offers very long opioid analgesia (up to 72 hours) and could be used for patients with swallow difficulties. Transdermal fentanyl patches are widely used for background pain.
Utilization of transbuccal route of administration creates very fast rescue medication for breakthrough pain. Transbuccal preparation is available as lozenges and effervescence tablet.
Electrophoretic fentanyl patch offers postoperative patient controlled analgesia system, which eliminates need of syringe driver and intravenous (or subcutaneous) contact. Fentanyl is administrated on patient request (pressing button) by transdermal electrophoresis.
Application of “magic bullets” (delivery systems) transformed simple molecule into number of different systems, which could be used widely in cancer and non-cancer pain management.

COXEN: A New Strategy For Predicting The Chemosensitivity Of Human Cancers And Its Application To Drug Discovery

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The U.S. National Cancer Institute has used a panel of 60 diverse human cancer cell lines (the NCI-60) to screen >100,000 chemical compounds for anticancer activity. However, not all important cancer types are included on the panel nor are drug responses on the panel predictive of clinical efficacy in patients. We thus asked whether it would be possible to identify common chemosensitivity biomarkers from that rich database to predict drug activity in cell types not included in the NCI-60 panel or, even further, clinical responses in patients with tumors. We address that challenge by developing a novel pharmacogenomic approach "Co-eXpression ExtrapolatioN" (COXEN), which can effectively identify concordant genomic chemosensitivity biomarkers between two independent expression profiling data sets, here extrapolating the genomic expression patterns of NCI-60 biomarkers with those of clinical tumors. Applying our COXEN approach in a prospective fashion, we predicted anticancer drug activities on completely independent bladder cancer, which is not included in the NCI-60 panel, and chemotherapeutic responses and survival of breast, bladder, and ovarian cancer patients treated with commonly used single and multi-agent chemotherapies. We also used COXEN for in silico screening of 45,545 compounds and identify a novel agent with superior growth inhibition activity against human bladder cancer.

Erythropoietin-Binding Protein And Its Antibodies For Possible Clinical Application

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Background: Hypertension (H), prevalent worldwide, is associated with high morbidity and mortality. Many investigators believe that genetic components are important in the etiology of H. In embryo transfer studies, genetic factors dominantly affected blood pressure (BP) ($P < 0.0001$) and its fluctuations (($P < 0.025$), when we transferred one-cell homozygous embryos into hypertensive or normotensive rats' oviduct, and pups were cross-suckled at birth. Erythropoietin (Epo) induced H is the most serious complication in Epo treatments (Rx). Uncontrollable BP rise in 1/3 of patients, end-organ damage and even death have resulted in the U.S. Food and Drug Administration "re-evaluating" the safety of Epo.
Method & Result: Human Epo-binding protein (Epo-bp) and anti-Epo-bp antibodies (αEpo-bp) were genetically engineered to test the adversity of Epoetin. Epo Rx increased hematocrit (Ht) markedly overall when compared to saline (S), Epo-bp, and αEpo-bp Rx (0.62 vs.0.43, 0.44, and 0.44, respectively) and at each of the 6 test times (all $P < 0.0001$). They had almost no effect on the Epo-induced Ht increase. Circadian BPs for Epo vs. S, Epo-bp, and αEpo-bp Rx were 136 ± 2 vs. 116 ± 2, 118 ± 2 and 117 ± 2 mm Hg, respectively (each $P < 0.0001$). Splenomegaly characterized each rat in the Epo Rx: in grams 1.58 in Epo vs. 0.86 in S, 0.89 in Epo-bp, and 0.85 in αEpo-bp (each $P < 0.0001$). Ligand-binding sites were detected using fluorescein-labeled Epo-bp & αEpo-bp in various blood progenitors. We developed diagnostic kits to detect Epo, Epo-bp and their antibodies to differentiate Epo- from EpoR-related diseases; Epo levels in serum & plasma: 25.4 ± 2; 24.2 ± 2; Epo-bp: 24.2 ± 2; 25.0 ± 1 mU/ml, respectively. Cell membrane proteins play a key role in cell-cell communications. Thus, exploring membrane protein polymorphisms and hormonal interactions may expand our knowledge of the normal and abnormal physiological process, and lead to the development of a new strategy in those Rx.
Conclusion: Epo-bp and αEpo-bp effectively eliminate Epo-induced H without affecting Ht. They are predicted to be therapeutic agents for hematopoietic malignancy, and used as diagnostic tools at test sites and quick detection of the athletic abuse of Epo as a doping agent, and as research tools, not only for Epo-EpoR-, but also for many other circulatory, vessel and tissue-related malignancies. Clinical implications of our materials are enormous and diverse, and provide hope for the effective Rx of those problems without damaging adversity, the perfect concept for the Magic Bullet envisioned by Dr. Ehrlich.

Systematic Discovery Of Novel Multi-Target Therapeutics: Finding The New Magic Bullets

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Background: Biological pathways fundamental to disease are highly complex and perturbation of a single molecular target often has little or no effect on a disease process due to redundancy and buffering of complex biological systems. Conversely, the perturbation of multiple targets in a critical disease pathway can simultaneously attack disease pathology on multiple fronts enabling treatment of disease through entirely new mechanisms of action. The rationale for a multi-targeted approach is especially applicable in oncology where pathogenesis often results from the complex intersection of many biological pathways.
Methods: To systematically discover multi-target mechanisms we generate a concentration matrix for each compound combination, capturing the combined activity of two compounds over a broad range of single agent concentrations. Analysis and quantitative scoring of concentration response matrices using multiple mathematical models allows insight into the biological mechanism of action of a drug combination and the discovery of novel therapeutic applications. Novel synergistic combination therapeutics are validated in secondary disease relevant *in vitro* and *in vivo* model systems and rapidly advanced to clinical proof of concept studies.
Results: We have discovered multiple unexpected synergistic combinations in oncology indications. In one example, the combination of an anti-parasitic agent, pentamidine and a phenothiazine anti-psychotic, chlorpromazine exerts an anti-proliferative effect through synergistic action on the mitotic targets KSP/Eg5 and PRL phosphatase. This combination synergizes *in vitro* and *in vivo* with the microtubule binding agents paclitaxel and vinorelbine, supporting a model where dual inhibition of mitotic kinesins and PRL phosphatases synergize in mitosis to inhibit tumor cell growth. In a second example, a multi-target mechanism screen in multiple myeloma cell lines has revealed novel and highly selective synergistic interactions between molecular pathways not previously known in multiple myeloma.
Conclusions: The systematic survey of multi-target mechanisms enables the discovery of interesting new biology, the definition of specific target pairs for therapeutic development and demonstrates the power of combination biology for drug discovery.

Magic Activity Of Beta-Lactam Antibiotics Against Intracellular Methicillin-Resistant *S. Aureus* (MRSA): Role Of Acidic pH

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Background. Early studies showed that MRSA strains become susceptible to β-lactams when they are exposed to acidic pH (pH ≤ 5.5) (Sabath et al., AAC, 1972). Because *S. aureus* survives in the phagolysosomes of macrophages (where the pH is in the acidic range), we have examined the intracellular susceptibility of MRSA strains to cloxacillin and meropenem.
Methods. Intracellular activity was determined against *S. aureus* phagocytosed by human THP-1 macrophages or skin keratinocytes. Expression of *mecA* and its regulatory genes was determined by RT-PCR. Cloning, purification of PBP2a, and Bocillin FL – PBP2a binding assay were performed as described earlier (Fuda et al, JBC, 2006; Lemaire et al, JBC, 2008).
Results and conclusions. MRSA ATCC 33591 phagocytosed by human THP-1 macrophages shows complete restoration of susceptibility to cloxacillin and meropenem, becoming indistinguishable from MSSA ATCC 25923 due to the acidic pH prevailing in the phagolysosomes (Lemaire et al, AAC, 2007). This influence of acidic pH was first ascribed to a diminished copy numbers of PBP2a (Hartman and Tomasz, J. Bacteriol., 1984), a unique transpeptidase that is poorly inhibited by beta-lactam antibiotics because of a closed conformation of its active site. However, we showed that growing bacteria at acidic pH (i) alters neither the expression of the PBP2a-encoding gene (mecA) nor that of its regulatory genes. We also found that MRSA grown and exposed to a radioactive penicillin ([^{14C}]penicillin) at acidic pH show a larger retention of radioactivity than if the bacteria has been grown at neutral pH (Lemaire et al, AAC, 2007). Concentrating our effort on a purified PBP2a, we showed that, at lower pH, PBP2a bind more avidly β-lactams and undergoes a conformational change (which is a crucial step for the opening of the active site) (Lemaire et al, JBC, 2008). In terms of mechanistic consequences, these variations were quite similar than those recently reported for ceftobiprole (a novel anti-MRSA cephalosporin inhibiting more efficiently PBP2a). Therefore, these observations argue that PBP2a is most likely evolved for its physiological function at pH 7.0 owing to its closed conformation, which is not maintained at acidic pH.

Dendritic Cell Immunotherapy Of Malignant Gliomas

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Background: Despite resent advances in radio-, chemotherapy and surgical treatment, the prognosis for patients with malignant glioma is still very poor. Therefore, the development of new treatments, such as immunotherapy is very important. Dendritic cells (DCs) are antigen presenting cells that play a central role in the initiation and modulation of immune response. In this study we investigated the safety and immunologic and clinical response of tumor lysate-pulsed DC therapy for patients with malignant glioma.
Methods: Thirty nine patients with anaplstic astrocytoma (AA, n=24) and glioblastoma (GB, n=15) were enrolled in this study. Controls represented the population of 80 patients (AA=47 and GB=33) who received surgical resection and radiation therapy. The patient's peripheral blood DC were generated with granulocyte macrophage colony-stimulating factor plus interferon-alpha during 4-5 days and pulsed with an autologous tumor lysate. DCs were used for the generation of CTL (that were inoculated in the cavity of rejected tumor) and the course of 4-6 biweekly subcutaneous vaccinations after radiation therapy.
Results: The protocol was well tolerated and was not associated with an evidence of toxicity or serious adverse effects. Sixty five percent of patients developed systemic immune response according to proliferation and positive delayed-type hypersensitivity skin test to autologous tumor lysate. Antigen (Ag)-specific response after 6 vaccinations increased in 10 folds (table). The level of 1- 2- and 3-year survival in this group was significantly higher in compare with controls (74 *vs* 52,5%, 61 *vs* 27,5 and 50 *vs* 19%, respectively). The most effect was observed in GB patients. A median survival in this group was 14 months *vs* 8 months in controls (p=0,003).

n=10	Before vaccination (cpm)	After 3 vaccination (cpm)	After 6 vaccination (cpm)
Without Ag	425 ± 74	321 ± 88	666 ± 145
Tumor Ag 0,01 mg/ml	663 ± 154 (1,65 ± 0,4)	363 ± 121 (1,3 ± 0,3)	5170 ± 1507 (10,9 ± 4,9) *Pu<0,05*
Control Ag 0,01 mg/ml	450 ± 57 (1,1 ± 0,35)	815 ± 79 (2,1 ± 0,6)	1050 ± 245 (1,36 ± 0,7)

Conclusion: Thus, our results showed the safety and clinical response of autologous tumor lysate-pulsed dendritic cell therapy for malignant glioma patients and the accumulation of antigen-specific immune response.

Exploring Anti-Microbial Herbs Of Chinese Medicine

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Background: The global increase in resistance to antimicrobial drugs, including the emergence of bacterial strains that are resistant to antibacterial agents, has created a public health problem of potentially crisis proportions. Thus, there is an urgent need for new antibacterial agents that are able to overcome multidrug-resistant mechanisms.
Aim: To identify herbs and their active components exhibiting inhibitory effect either on the growth or the bacterial resistance mechanism.
Methods: Traditional Chinese Medicinal (TCM) herbs, which are commonly employed in the treatment of bacterial infections, were identified. Potential herbs were then extracted using a standardized protocol. Assays for screening were prepared using a Biomek 3000 (Beckman Coulter) liquid handling system, and microtitration plates. Five µl of extract with desired concentration was added to each well of the 96 square-well plate containing Mueller-Hinton medium and 106 CFU/ml of the bacteria. Plates were then transferred to the integrated DTX 880 Multimode detector for incubation (37°C) and growth was monitored for 48 hours by measuring the absorbance under O.D. 600nm. The extract was considered very active if there was no bacterial growth after 24hrs incubation and as active if bacterial growth was more than 10% of the negative control.
Results: A total of 10 TCM herbs were subjected to preliminary antibacterial screening against 3 clinical and pathogenic bacterial strains of Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923) and Methicillin-resistant S. aureus (ATCC BAA- 43). The results indicated that out of 10 herbs screened, ethanol extract (100µg/ml) of Caulis Sargentodoxae *(Sargentodoxa cuneata)* and Coretx Lycii *(Lycii chinensis)* showed broad-spectrum antibacterial activity against both S. aureus and MRSA compared to aqueous extract. Percentage inhibition of bacterial growth was 88% - 90% and 90 – 100% for both C. Sargentodoxae and C. Lycii respectively ($p<0.001$). However, extracts from these two herbs exhibited little or no activity, against E. coli.
Conclusions: We have validated the protocol for screening bioassay for evaluating the antibacterial properties of herbal extracts. Our preliminary results suggest that the ethanolic extracts of Caulis Sargentodoxae and Coretx Lycii have antibacterial activity. Although further studies are needed to determine safety and clinical efficacy, these effective extracts may prove to be clinically useful in the treatment of multidrug resistant bacterial strains.

Signal Transduction Therapy Of Cancer

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Background: Signal transduction therapy was introduced into cancer therapeutics in the early 1990s after the first tyrphostins and anti-Her2 antibodies were developed. Currently there are a few antibodies like Herceptin, Avastin and Erbitux in the clinic as well as eight tyrosine phosphorylation inhibitors.
Methods: The design and synthesis of novel protein kinase inhibitors that are ATP non-competitive inhibitors will be presented. We shall demonstrate the successful use of a combination of an EGFR kinase inhibitor with our novel PKB/Akt inhibitor and a DNA damaging agent to strongly inhibit glioblastoma, which overexpreses EGFR and lacks PTEN. We shall also present the design, synthesis and performance of a chemical vector homing for the EGF receptor. The vector is loaded with PolyIC and due to the ability of the EGFR to internalize the vector; PolyIC is inserted into the cell, eliciting a strong anti-tumor response. Here we shall specifically discuss an EGFR homing vector carrying PolyIC.
Results: In view of the success of targeted cancer therapy since the early studies on tyrphostins (1988) we have enhanced our efforts to improve the performance of targeted therapies by following new paradigms. In this talk I will present three ongoing studies in our laboratory (1) the development of novel substrate competitive PKB/Akt inhibitors and how they perform in the treatment of prostate cancer and brain cancer in nude mice. (2) The development of novel allosteric IGF1R inhibitors that lead to the degradation of the IRS proteins and their highly potent in vivo efficacy against disseminated ovary cancer, breast cancer, and prostate cancer in nude mice and (3) the eradication of EGFR over-expressing disseminated tumors by EGFR targeted long chain dsRNA (PolyIC). This approach is highly effective due to the targeted "bystander" effect induced by the PolyIC that is inserted selectively into the EGFR over-expressing tumor cells. This is by far the most effective signal transduction therapy regimen in the treatment of EGFR over-expressing tumors, in experimental animals.
Conclusions: (1) Non-ATP competitive protein kinase inhibitors are highly effective agents and may be used with success to treat various cancers. (2) A chemical vector homing to a receptor, which is over-expressed in tumors and which is capable of internalization can induce complete eradication of the tumor if loaded with PolyIC. This approach can be in principle be utilized with many receptors as a target, where the example given here is an EGFR homing vector.

Molecular Targeting of the *bcl-2* Oncogene for Staging and Therapy of B-Cell Lymphoma

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Background: The *B-cell lymphoma/leukemia-2* (*bcl-2*) oncogene is a dominant inhibitor of apoptosis, correlating with resistance to radiation and chemotherapy, high relapse rate, and poor survival in non-Hodgkin's B-cell lymphoma (NHL). NHL also expresses type 2 somatostatin receptors (SSTR2) in 87% of cases, making it attractive for delivery of intracellular tumor-targeting agents.
Methods: A *bcl-2* antisense peptide nucleic acid (PNA) conjugated to a SSTR2-targeting peptide, anti-*bcl-2*-Tyr^3-octreotate, was evaluated for ^{111}In gamma scintigraphy and single photon emission computed tomography (SPECT), ^{64}Cu positron emission tomography (PET), and ^{177}Lu targeted radiotherapy (TRT) in NHL cells in culture, mouse models of human NHL, and dogs with spontaneously occurring NHL. SCID mice bearing bcl-2 mRNA-positive Mec-1 (n = 3) or bcl-2 mRNA-negative Ramos (n = 3) xenografts were used for ^{111}In microSPECT or ^{64}Cu microPET imaging. The ^{111}In conjugate was also used for gamma scintigraphy of canine NHL patients (n = 15). The ^{177}Lu conjugate was evaluated in vitro for TRT in Mec-1 cells (n = 3).
Results: Incubation of Mec-1 cells with anti-*bcl-2*-Tyr^3-octreotate showed a 51% decrease in bcl-2 protein synthesis, suggesting that the target mRNA function had been perturbed by a specific antisense effect. Both ^{111}In microSPECT and ^{64}Cu microPET could detect Mec-1 tumors, but not Ramos tumors, in SCID mice ($p < 0.05$). Gamma scintigraphy demonstrated the utility of the ^{111}In conjugate for molecular staging of *bcl-2* in canine NHL. In vitro Mec-1 cell studies showed that the ^{177}Lu conjugate had at least an additive effect on cell viability, compared to controls for targeted radioactivity and *bcl-2* antisense activity.
Conclusions: 1) Imaging studies demonstrated that ^{111}In- and ^{64}Cu-anti-*bcl-2*-Tyr^3-octreotate were specific for bcl-2 mRNA-positive NHL xenografts. 2) Imaging of canine NHL established *bcl-2* expression as a clinical and molecular model relevant to human disease. 3) TRT studies of the ^{177}Lu conjugate in Mec-1 cells demonstrated down-regulation of *bcl-2* with radiation insult, creating a NHL therapy agent acting through two targeted anti-tumor mechanisms.

Development of Novel χ-Conopeptide Inhibitors of the Norepinephrine Transporter for the Treatment of Severe Pain

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Background: Venoms are a valuable source of novel bioactive peptides. The venom of ~500 species of cone snails contain 500,000+ small bioactive peptides (venom peptides) that target specific ion channels, transporters and receptors. A number of classes of conotoxins have emerged with therapeutic potential in the treatment of neuropathic and inflammatory pain states, including the χ-conotoxins that inhibit the norepinephrine transporter. The aim of the present study was to evaluate a series of χ-conotoxins for their ability to inhibit the norepinephrine transporter (NET) and reverse pain behaviors in a rat model of neuropathic pain.
Methods: χ-Conopeptide analogues were assembled by solid phase peptide synthesis and were assessed for their ability to inhibit [^{3}H]-NE uptake through human NET. Selected analogues were further evaluated for target specificity and ability to reverse allodynia in a rat model of neuropathic pain when delivered intrathecally.
Results: Over 300 χ-conotoxin analogues were synthesized. Their potency to inhibit NE uptake ranged from >80-fold enhanced to >1000-fold reduced compared with the parent conopeptide, helping define the χ-conotoxin pharmacophore. Sixteen analogues were selected across the series of active leads and tested in a neuropathic pain model. Several potent analogues produced less than expected efficacy and one related series produced side effects of unknown origin. Based on superior animal efficacy, side effect profile and chemical stability, Xen2174 was progressed into the clinic. An open-label clinical study of pain in oncology patients revealed that (i) a single intrathecal bolus dose of Xen2174 was safe and well-tolerated up to a dose of 30 mg, and (ii) some patients experienced multiple-day pain relief.
Conclusions: These studies indicate an important role of the norepinephrine transporter in controlling the effects of norepinephrine released from spinal descending inhibitory pathways to reduce pain states in rats and possibly in humans.

Antibody-Therapeutic Targeting Of TolC For Growth-Combating Of Antibiotic-Resistant *Escherichia Coli*

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Background: Bacterial resistance to an antibiotic may result from survival in a suddenly strong antibiotic or in sub-minimum inhibitory concentration of the drug. Their shared proteins responsible for the resistance should be potential targets for designing of new drugs to inhibit the growth of the antibiotic-resistant bacteria. Aims: 1) To identify sharedly altered outer membrane proteins (OM proteins) that are responsible for chloramphenical (CAP)-resistant *Escherichia coli* and for survival in medium with suddenly strong CAP treatment. 2) To develop a novel method of specific antibody combating bacterial growth based on these shared OM proteins.
Methods: Comparative sub-proteomic methodologies were used to identify differentially expressed OM proteomes from *E. coli* respond to suddenly increased CAP treatment and from CAP-resistant *E. coli* selected from survivals after ten passages of subculture with the sub-inhibitory concentration of CAP. After the shared OM proteome responsible for CAP between the two ways of the exposures to the antibiotic treatment were determined, their capacity in antibiotic resistance was further investigated by their mutants. A specific antibody combating growth assay was developed to inhibit the activity of CAP-resistant OM proteins. The inoculums of CAP-R, CAP-R-O, □*tolC*, □*ompC*, □*ompT* and □*ompW* were separately cultured in 5 mL fresh LB medium at 37 ^{0}C overnight, and then the cultures were diluted 1:100 into 5 ml fresh LB medium to obtain the desired cell density (OD600nm=0.5, 10^8 CFU/ml). Pellet from 200 μL of each of six cultures was obtained by centrifugation at 9000×g for 4 min and was separately incubated with 50μl of rabbit pre-immune serum and immune serum against TolC, OmpC, OmpT or OmpW at 37 ^{0}C for 1h. After centrifugation at 9000×g for 4 min, the bacteria were suspended in 200 μL fresh LB medium and then were diluted 1:1000 into 5 mL LB medium without or with 1/8 MIC CAP. These cultures were incubated at 37 ^{0}C for 9 h and were measured at OD600nm for survival capacity.
Results: Six differential OM proteins and an unknown location protein were determined to be sharedly CAP-resistant-related proteins with the use of 2-DE/MS, Western blotting and gene mutant methods, in which TolC, OmpT, OmpC and OmpW were critical altered proteins and potential targets for the designation of the new drugs. Furthermore, only anti-TolC showed a very significant inhibition on bacterial growth in medium with CAP when antisera to TolC, OmpC, OmpT and OmpW were separately applied. The growth of CAP-resistant *E. coli* and its original strain was completely inhibited when they bound with anti-TolC and survived in 1/8 MIC of CAP, which was equal to behavior of □*tolC* when it did in the same concentration of the antibiotic.
Conclusions: 1) Bacterial growth can be combated using the antibody specific to TolC, suggesting a novel insight into therapy to infection by antibiotic-resistance bacteria. 2) Combination therapy involving antibiotics that enhance the expression of an antibody target could be far more effective than either drug alone, which gives a novel insight into therapy to infection by antibiotic-resistant bacteria. This work was sponsored by grants from NSFC project 30530610, Guangzhou Key Project 2006Z3-E0251 and Guangdong NSF key project (7117645)..

Funding New Technologies For The Development Of Seasonal And Pandemic Influenza Vaccines

LI S

Biomedical Advanced Research and Development Authority, US Department of Health and Human Services

Influenza viruses, both seasonal and pandemic, pose an ongoing threat to global health. Vaccines remain the best means of controlling influenza infection and spread, but the current worldwide capacity for seasonal influenza vaccine production falls far short of the capacity needed to provide sufficient vaccine for mass immunization in the event of a pandemic outbreak. To increase the supply of seasonal vaccine and the surge capacity for producing pandemic vaccine, the U.S. Government has established programs to facilitate the development of new technologies for enhancing the domestic and international influenza vaccine manufacturing infrastructure. Three major initiatives have been undertaken to support advanced development of: i) cell culture-based influenza vaccines; ii) novel dose-sparing adjuvant technologies for pandemic influenza vaccines; and iii) recombinant influenza vaccines. Funding has been provided under these programs for process development, clinical studies, establishment of manufacturing facilities, and other activities leading to licensure of the vaccines by U.S. Food and Drug Administration. The U.S. Government has awarded six contracts for the development of cell-based influenza vaccines. The cell-based technologies will complement the current licensed egg-based manufacturing technology to meet the USG's stated goal of producing 450 million doses of vaccines within six months. Three contracts have been awarded for the development of novel adjuvants that would allow antigen-sparing in H5N1 vaccines. As the non-adjuvanted H5N1 vaccine is poorly immunogenic, and thus requires high doses of antigen to be effective in humans, development of these adjuvants may be critical for meeting potential pandemic needs. The successful conclusion of these programs will fundamentally alter the U.S. and global influenza vaccine manufacturing base and provide increased vaccine production capacity for pandemic preparedness.

Pharmacogenomic Targeting Of Ehrlich's Magic Bullet

LI-WAN-PO A

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Background: Paul Ehrlich focused on targeting microbial invaders and renegade (cancer) cells. Despite his valiant efforts, both the enemies within, and the, invaders have proved resilient. However, considerable progress has been made since and we demonstrate the wide applicability of his vision and approach.
Methods: We surveyed drugs in current and investigational use, and highlight the dramatic progress made through application of Ehrlichian principles of drug discovery and optimization.
Results: Ehrlich, the visionary, was visual in his approach. His genius was to recognize that selective staining of microbes and cancer cells highlighted a route to drug selectivity. We still use the same approach but probe at a deeper genomic level, and on a vastly wider scale using gene expression microarrays. Ehrlich used both small molecules and macromolecules in his armamentarium. We maintain this mix to include an array of selective small molecules for targeting specific enzymes (e.g. imatinib) and disease pathways, and highly selective macromolecular drugs (e.g. monoclonal antibodies) for virtually every degenerative disease. Moreover, we can now identify the enemy with much greater precision (e.g. fine molecular classification of clinically similar diseases, and identification of resistance genes and epitopes to develop effective vaccines against enemies, both seen and unseen). Use of combinations of magic bullets causes less collateral damage (e.g. infections, rheumatoid arthritis), and lowers the risk of resistance. Unravelling more of the mysteries of our DNA self, helps us develop new magic bullets (e.g. siRNA), and new shuttles for our wounded troops (e.g. gene vectors) and sometimes to act as decoys against renegades (e.g. suicide gene therapy). Learning how to navigate in and out of tunnels (transmembrane transporter pharmacogenetics) ensures that our magic bullets are aimed more precisely. We illustrate these visually in our presentation.
Conclusions:
Ehrlich's magic bullet has undergone considerable development. With our ageing populations, Ehrlich's disciples have to contend with an increasing number of enemies from within as well as the persistently challenging invaders. It is of course ordained that the enemy will always win in the end but new Ehrlichian weaponry is allowing us to prolong life and improve its quality.

Enhancement Of Radiation Or Chemotherapeutic Effects Of Para-Aminobenoic Acid (PABA) And A Novel Analog On Melanoma: Preclinical Studies And Phase I Studies

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Background: B16F10 melanoma cells cultured in media containing PABA resulted in a depigmentation and inhibition of tyrosinase activity relative to cells grown in PABA deficient media. Following PABA's potentiation of radiation or paclitaxel cytotoxicity, mechanistic studies showed inhibition of cell cycle arrest by up-regulation of CDC25A and down-regulation of $p21^{CIP1}$ and BRCA2. A Phase I study of combination PABA, Paclitaxel (P) and carboplatin(C) in metastatic melanoma patients who had failed first-line therapy was initiated. Structure-activity studies utilizing tyrosinase activity identified C45, as a more potent PABA analog.
Methods: Cell culture and murine models assessed the effects of PABA and C45 on melanoma tumor growth and gene expression. Cell cycle modulation and potentiation of cytotoxicity was greatest for P. We then enrolled patients (pts) with metastatic melanoma who had failed at least one prior chemotherapy regimen to receive PABA in combination with C and dose-escalated P. Measurement of plasma pharmacokinetics (PK) of PABA prior to, and 24 hours (h) after carboplatin/paclitaxel were obtained as well as PK of P after PABA administration.
Results: C45 showed modulation of cellular proliferation in Lewis Lung and B16F10 melanoma. It potentiated the in-vivo activity of P, Temozolomide and Alimta and external beam radiation in M21 and B16F10 models. In the phase I, 19 pts were enrolled with 18 available for assessment. One grade IV neutropenia was seen and no dose-limiting toxicities even at doses of C AUC 5, and P 175 mg/m2 that are in common use. PABA PK for dose levels I-V showed a mean C_{max} of 14 μg/ml $\pm$ 7.9 μg/ml at 0.5 h, and a mean elimination half-life of 2 h. In the presence of P, the mean half-life of PABA was 4.6 h. Four partial responses were seen and 2 had stable disease, with duration of response ranging from 12-32 weeks.
Conclusions: PABA is safely administered in combination with full dose P and C in pts with refractory metastatic melanoma. Our enhanced therapeutic activity with low toxicity, justifies a phase II efficacy trial. Studies are continuing with the evaluation of the dose response and pharmacokinetics of the PABA analog, C45.

Authors' disclosure statement (not counting towards the character count):
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Advantages of Multiple Drug Interactions: Combinatorial Treatments Using Neural Networks

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Background: There are few Magic Bullets since drugs designed for one specific effect also interact with many other molecules in a cell. Instead of a disadvantage, we can turn this network of interactions into an advantage. If we can infer enough information about this network, from a limited set of experiments, we can predict which combinations of drugs will interact with each other, in just the right way, to have the most targeted effect with the fewest side effects. We call this approach Combinatorial Multi-Component Thearpy (CMCT).
Methods: To illustrate this approach we constructed model test networks of linear or non-linear drug interactions. We then trained an artificial neural network (ANN) on a limited data set of drug inputs presented one-at-time and pairs-at-a-time and their output effect predicted by these models. We optimized the performance of the ANN by using only one output, softening the transfer function between the input and hidden layer, using four times as many units in the hidden layer as the input, and taking the logarithm of the output values.
Results: For the model test networks, the ANN, trained on only very limited data, accurately computed the output effect for different combinations of input drugs. For example, 99% of the outputs had errors of less than 10% for all 32,768 combinations of 15 inputs that are either {0,1} in a highly nonlinear model of drug interactions.
Conclusions: These test results suggest that this CMCT approach may be of value in determining how combinations of drugs can be effectively used to achieve specific therapeutic results.

Ecstasy – The Pharmacology of Happiness

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Millions of young people consume „Ecstasy“ (MDMA), indicating that MDMA has strong rewarding effects. MDMA induces a state of well-being, elevate mood, moderate psychomotor stimulation, few perceptual changes and little anxiety.

MDMA acts at the presynaptic monoamine transporter and releases serotonin, dopamine, and norepinephrine by reversing the transport of these monoamines. While the neurochemical effects of MDMA have been well described in preclinical models, it is less clear how the neurochemistry translates into to the psychotropic effects in humans. The rewarding effects of drugs of abuse are commonly attributed to the release of dopamine in the mesolimbic system. Indeed, pharmacological blockade of dopaminergic D_2 receptors with haloperidol attenuated the positive mood effects of MDMA and produced increased adverse effects including anxiety. Serotonin uptake inhibitors (SSRIs) decrease MDMA-induced serotonin release by blocking the interaction of MDMA with the serotonin uptake site. In humans, administration of an SSRI prior to MDMA markedly decreased all subjective and physiological effects of MDMA including its positive effects, negative/adverse effects, the slight perceptual changes and the increase in blood pressure and heart rate. Further, blockade of the postsynaptic serotonergic 5-HT_2 receptors selectively attenuated MDMA-induced perceptual chances. Together these results indicate that the MDMA-induced effects are overall due to release of endogenous serotonin with contributing effects of dopamine release to positive mood. MDMA-induced hallucinogen-like perceptual changes can be linked to 5-HT_2 receptor stimulation.

Ecstasy use is associated with serious adverse effects including hyperthermia, liver failure, hyponatremic brain edema and cardiovascular complications. Furthermore, there are concerns that heavy chronic Ecstasy use may lead to lasting cognitive impairment due to serotonergic neurotoxicity.

So far, the magic Ecstasy pills induce short-term happiness but severe adverse effects and potentially persisting cognitive effects are a high prize to pay. Recent rodent studies indicate that the Alzheimer treatment memantine prevented MDMA-induced neurotoxicity and cognitive deficits. The question is open whether the magic bullet – the safe pill for happiness – will contain a psychostimulant and a neuroprotective treatment for Alzheimer's disease.

Engineering Human Bak Proteoliposomes: a New Approach for the Treatment of Glioblastoma

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Background. Specific delivery by nanoparticles of functional therapeutic proteins into targeted living cells is one of the most promising strategies for cancer treatment. The use of liposomes in the delivery of therapeutic membrane proteins directly into cells have not been tested because methods for producing membrane proteins and functional proteoliposomes are still difficult to carry out.
Methods. One-step expression of human Bak and mutant Bak (Bak□BH3) proteoliposomes was tested using an optimized *E. coli* cell-free synthesis system in the presence of PEGylated liposomes. Subcutaneous inoculations of brain tumor (GL 26) on C/57/BL mice were performed into the rear left flanks and on day 20 post cell injection the tumor reaches 1mm^3, the tumor-bearing mice were injected with 75 µg of recombinant Bak or Bak□BH3 proteoliposomes. The mice were monitored for tumor regression and survival rate.
Results. We demonstrate that the yields of pure Bak proteoliposomes are up to 2 mg/ml of reaction. Pre-clinical experiments indicate a significant regression in the tumor mass when mice were treated with lipo-Bak from day 15 post-injections and total absence of tumor in 60% of the mice treated after 30 days. The same treatment with lipoBak□BH3 shows a plateau in the tumor growth followed by an exponential proliferation of the tumor. Survival analysis indicates that 60% of mice treated with lipoBak were still alive after 80 days after injection.
Conclusions Here we report the therapeutic effect of long-circulating Bak proteoliposomes produced with an innovative cell-free expression system. We can conclude that:
1) Cell free expression system is a powerful tool to produce proteoliposomes with inserted therapeutic proteins (hBak).
2) In vitro experiments on different cancer cell lines demonstrate that lipo-Bak is able to be internalized and to exert an apoptotic effect.
3) Results on mice subcutaneous glioblastoma model show a survival of 60% of the treated mice with a total regression of the tumor.
Considering this proof of concept, we are convinced that engineering human Bak proteoliposomes can be an effective new approach for the treatment of glioblastoma.

Peripherally Administered TrkB Agonists Cause Appetite Enhancement and Weight Gain in Non-Human Primates

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Background: Brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT4), ligands of receptor tyrosine kinase trkB, are anorexigenic when administered peripherally or centrally in mice. We hypothesized that like rodents, peripheral or central administration of TrkB agonists could reduce body weight and food intake in non-human primate species. If true, TrkB agonism may be a therapeutic approach for human obesity.
Methods: 8 rhesus monkeys received a dose escalation of BDNF (n=4) or NT4 (n=4) by intracerebroventricular (ICV) delivery. 24 cynomolgus macaques were administered with NT4 either daily intravenously (IV), or daily subcutaneously (SC), or with a TrkB agonist antibody twice weekly IV. 6 obese baboons were given NT4 (n=3) or vehicle (n=3) daily IV. These baboons were later administered NT4 (n=3) or vehicle (n=3) IV twice weekly.
Results: ICV administration of NT4 or BDNF into rhesus monkeys resulted in a dose-dependent suppression of food intake. Daily SC dosing of 2mg/kg of NT4, or IV dosing of 2mg/kg in cynos monkeys resulted in a 2 to 3-fold increase in daily food intake, and a 1.6 to 2.3-fold increase in cumulative food intake respectively. Daily SC or IV injections resulted in a 16% (SC for 21 days, $p<0.001$)) or 33% (IV for 30days, $p<0.001$) increase of body weight respectively. Twice a week IV dosing (21 days) of the TrkB agonist antibody (5mg/kg) in cynos monkeys resulted in a 40% increase in cumulative food intake ($p<0.001$) and a 10% increase in body weight ($p<0.01$). Obese baboons given daily IV injections of NT4 (2mg/kg) increased their daily food intake by 2 to 3-fold, and their cumulative food intake (25 days) by 2.5 fold. Body weight increased by 16% ($p<0.001$).
Conclusions: 1) We observed a novel orexigenic response to peripheral administration of TrkB agonists, that is contrary to the anorexigenic response of peripheral or central TrkB agonism in mice. 2) Peripheral administration of NT4 was well-tolerated, suggesting that TrkB agonism could be a feasible therapeutic for anorexia or cachexia.

Hyaluronan-Mediated Transformation And Relapse Of Prostate Cancer

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INTRODUCTION: Interaction between extracellular matrices (ECM) and cancer cell receptors frequently alters signal transduction pathways, which lead to malignant transformation and metastasis. Hyaluronan (HA), an ECM tumor promoter and enhancer, is synthesized by stromal fibroblasts in response to paracrine factors produced by tumor cells. This type of tumor-stromal interaction plays a crucial role in the stimulation and promotion of cancer cell tumorigenecity. However, the molecular mechanism remains unclear.

MATERIALS &METHODS: Human microarray analysis and immunodetection in human CaP tissue arrays were used to screen differentially expressed oncogene markers from patients with various stages of prostate cancer (CaP). *In vitro* tumorigenecity assays were performed to evaluate the loss-of-oncogene function, including cell-cycle flow cytometry (cell proliferation), cell invasion chamber (migration and invasion), and adhesion to a human bone marrow endothelial cell (hBMEC) monolayer (metastasis).

RESULTS: We observed that HA-mediated CD168, a receptor for HA-mediated motility (RHAMM), and its downstream signal molecules, including ROCK1, Gab-1, PI3K•p110α and eIF4E, promote the malignant progression of hormone-refractory CaP (*Carcinogenesis* 28: 310-320, 2007). In normal prostate, androgen receptor (AR) serves as a tumor suppressor against the HA-stimulated CD168 signaling by binding and inactivating CD168 (*Carcinogenesis* 29: 282-290, 2008). AR is also found to regulate the transcription of CD168 mRNA in the presence of androgen. The results of *in-vitro* tumorigenecity assays further showed that CaP cells with deficiency or mutation of AR significantly increase the malignancy of cancer tumorigenecity in terms of cell proliferation, cell invasion and metastasis into the hBMEC monolayer. The expression of mir-146 against the key kinase ROCK1 of the HA–CD168 signaling pathway can reverse the HA-stimulated malignancy (*RNA* 14: 417-424, 2008).

CONCLUSION: HA activates the signal transduction cascade of CD168–ROCK1–PI3K–eIF4E in CaP, which can be prevented by ROCK depletion using mir-146. Therefore, our study suggests that the combination of current cancer therapy with an anti-ROCK agent may lead to beneficial results in preventing HA-mediated cancer transformation and relapse.

Verotoxin (Shiga toxin) binding to its receptor glycolipid, globotriaosyl ceramide, provides a new antineoplastic tool and physiologically-based approaches to tumour cell drug resistance

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Background Verotoxin 1 (Shiga toxin) kills cells expressing its receptor glycosphingolipid(GSL), globotriaosyl ceramide (Gb_3) expressed within detergent resistant plasma membrane (DRM) domains. Gb_3 is upregulated in many human cancers including breast, ovarian, colon, glioblastoma, meningioma, renal, testicular. Although Gb_3 is expressed on a few normal tissues these are not targeted in a primate model, suggesting that Gb_3 may not be in DRM in such cases. Intratumoral VT1 injection reduces growth rate and eliminates human Gb_3 positive tumors xenografts(astrocytoma, meningioma, colon and renal carcinomas) grown in mice. Gb_3 is also expressed in tumor neovasculature, indicating that VT1 has antineoplastic and antiangiogenic activity. Primate studies established a safe VT1 dosage for clinical trials.
Methods MDR1 transfected MDCK cells were used to assess MDR1 inhibition and link between MDR1 processing and GSL biosynthesis and polarized C2BBe1 gastrointestinal epithelial cells use to measure MDR1 mediated drug fluxes.
Results Gb_3 is particularly elevated in MDR1 expressing drug resistant tumour variants which led us to determine that MDR1 is a Golgi GSL flippase, involved in neutral GSL biosynthesis [1]. Inhibition of GSL biosynthesis prevents cell surface MDR1 expression in cell lines from drug resistant tumors, though intracellular MDR1 accumulates. Cell surface MDR1 showed significant colocalization with Gb_3 as monitored by VT1 binding. MDR1 is found within DRMs and treatment of drug resistant cells with VT1, or its receptor binding B subunit, internalized Gb_3 (in DRMs) to prevent MDR1–mediated rhodamine efflux. Thus the glycolipid environment of MDR1 is important to function and cell surface trafficking. AdamantylGb$_3$ is a soluble Gb_3 mimic we designed which competes for VT1-Gb_3 binding. AdamantylGb$_3$ was found to bind deep between the 6th and 7th membrane-spanning □-helixes of MDR1, and proved the first physiologically-based inhibitor of MDR1 [2]. MDR1-mediated rhodamine efflux was prevented and adamantylGb$_3$ reversed cell resistance to vinblastin. Gastrointestinal MDR1 also reduces oral drug bioavailability. MDR1-mediated digoxin and vinblastin efflux in human intestinal epithelial cells was prevented by adamantylGb$_3$.
Conclusion Gb_3 provides an antineoplastic target and new insight into tumour drug resistance and drug oral bioavailability.

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Dodging the bullets: an update on the multi-drug efflux pump P-glycoprotein

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Background: Human P-glycoprotein (Pgp, ABCB1) is a clinically-relevant drug exporter of the ATP Binding Cassette transporter family. The protein has two transmembrane domains (TMD) that contain the drug binding sites, and two nucleotide binding domains (NBD) that bind and hydrolyse ATP to drive the drug transport cycle. Structural studies on bacterial homologues indicate that the two NBDs interface to form two composite sites of highly conserved motifs to co-ordinate ATP. Each site comprises a Walker A and B motif, stacking aromatic and H-loop of the core subdomain of one NBD, and the ABC Signature and D-loop of the α-helical subdomain of the second. The highly conserved glutamine of the Q-loop also contacts the γ-phosphate of ATP via a water molecule. The Q-loop links the core and α-helical subdomains of the NBD, and also interdigitates with the TMDs.
Methods: We systematically examined the importance of these motifs in the function of Pgp by mutating the most highly conserved residue in each motif in either or both NBDs. The proteins were expressed in HEK293T cells and measured surface expression and drug efflux in real time by flow cytometry. Drug-stimulated ATPase activity of purified Pgp was measured by colorimetric assay.
Results: Single mutations introduced into the Walker A or B motifs render the protein virtually inactive. However, for the stacking aromatic, the ABC signature and the D-, H- and Q-loop motifs, mutation of one NBD has no, or minimal effect, on drug transport, but mutation of the motif in both NBDs has a strong, synergistic, negative effect. The high level of activity in the single Q-loop mutants was particularly surprising as data from mouse Pgp published previously suggests that these should be severely debilitated. However, on purification, the single Q-loop mutants of human Pgp exhibit <10% of the drug-stimulated ATPase activity of wild type Pgp and the double mutant was inactive.
Conclusions: The emerging picture is that canonical Walker A and B motifs are essential for drug transport, however, there is redundancy in the mechanism for the stacking aromatic, the ABC signature and the D-, H- and Q-loop motifs. In the absence of either Q-loop glutamine, Pgp appears sensitive to detergent. Together with structural data, this suggests that the Q-loop is the fulcrum of the molecular mechanism and an important conduit for energy transduction between domains.

Authors' disclosure statement:
The abstract describes unpublished data

Fluorescent Biosensors to Detect Magic Bullets against Multi-Drug Resistant Bacteria

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Background: Rising antimicrobial resistance of major pathogens limits therapeutic options. Identifying novel antimicrobial targets through genomic-derived, target-based approaches have widely failed in the past decades. Whole-cell bacterial biosensors with promoter-inducible fluorescent reporters provide an attractive method to discover novel drugs. However, there is a bottleneck in finding suitable pathway-specific biosensors because of insufficient specificity and poor signal to noise ratio.
Methods: *E. coli* promoter trap libraries were constructed containing random bacterial DNA in front of the reporter gene green fluorescent protein. Several fluorescent biosensors that report specific disturbance in cell wall and protein biosynthesis pathways were found. An optimal assay protocol for screening in a 96-well microtiter plate format was developed.
Results: These biosensors react only to inhibitors that reach significant concentrations in live bacteria and not to unspecific stresses. We optimized these biosensors for HTS screening in a 96-well plate format.
In comparison to luciferase or beta-galactosidase based biosensors, we can combine in a single well up to six fluorescent biosensors expressing different variants of GFP and simultaneously detect distinctive responses of each individual biosensor in the composite using automated multi-color FACS analysis. Our biosensors are more sensitive than growth assays and highly specific for well-defined targets. They are compatible for high-throughput-screening with a LSR2 flow cytometer with a high throughput sampler. The system measures 13 parameters per well. Automated high-content analysis records then individual information about bacterial growth, cell wall permeability and fluorescent protein expression in up to six different biosensors incubated with compounds.
Conclusions: A new sophisticated screening system was developed. The system can detect specific groups of antimicrobial compounds. Incubation times are short and only low compound concentrations are needed. This opens up new perspectives for antimicrobial screening.

Alpha-1-antitrypsin and IgA in Serial Meconium and Faeces for Date Newborn`s Faeces Formed during Intrauterine and Extrauterine Maturation.

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Background: Meconium is a series of layers formed in the foetal intestine from the 12th week of gestation. High content of meconial alpha-1-antitrypsin (AAT) , decreasing within the first days of extrauterine life appears to reflect the meconium clearance of the gut. At birth IgA is not present in the meconium and breast-fed infants receive this antibody postnatally with human milk.
Methods*:* 24 healthy breast-fed newborns were studied prospectively during the first 4 days of postnatal life. AAT and IgA concentrations in meconial and faecal samples and IgA concentration in mother`s milk taken on the third day after delivery were determined by radial immunodiffusion.
Results*:* The medians (range) of AAT concentrations in milligrams per gram of dry meconium or faeces were: 68,8 (29,2-138,4) (day 1), 56,9 (30,8-112,8) (day 2), 26,2 (6,8-80,7) (day 3), and 6,6 (1,4-27,1) (day 4). The median (range) of IgA concentration in mothers' milk was 715 mg/dl (420-890). IgA was absent in meconium portions from the first day of life while on the successive days the medians (range) of IgA concentration in mg/g dry mass of meconium and faeces were as follows: 0 (0-2,90) (day 2), 2,50 (1,10-9,60) (day 3), 7,05 (4,10-30,60) (day 4). On the day 4 of extrauterine life a negative correlation was found between AAT and IgA concentrations in faeces of the newborns (r= - 0,46).
Conclusions*:* 1) Analysis of the systematic decrease in AAT and increase of IgA concentration in serial portions of meconium and faeces over the first days of extrauterine life of breast –fed newborns can be a new marker for identifying meconium formed in utero. 2) Taking into account, that the use of illicit and legal drugs during pregnancy is common, meconium formed in utero can be a new clinical material for identifying exposure of infants to a number of illicit and legal drugs during 12-40 weeks of gestation.

Antibiotic "Magic Bullets": The Promise and the Reality of the Past Thirty Years

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Background: Antibiotics, vaccines, and biologic mediators have been true "magic bullets" against infections over the past 30 years. However, benefit has been tempered by toxicity, side effects, cost, and rising antimicrobial resistance. Even their successes have led to physician inappropriate use and public complacency about infectious diseases.
Methods: Local surveys of infections and resistant microbe prevalence was compared with healthcare professionals' attitudes on antibiotic and vaccine use. This was correlated with national and global data searching for broad trends. Literature was reviewed for approaches to countering resistance development and minimizing adverse events while promoting appropriate antibiotic use.
Results: In Philadelphia, pneumococcal penicillin resistance has risen from <10% to 29% over three decades; macrolide resistance is now >35%. Extended spectrum cephaloporins must be used carefully against enteric gram-negative bacilli due to extended spectrum beta-lactamases. Fluoroquinolones are much less effective against *Pseudomonas a.* than 30 years ago (0% resistance vs. 38% now). Vaccine uptake in children is only 80% currently, and some parents/patients decline inoculations as "not needed" (measles, mumps) or "not effective or dangerous" (influenza). *Clostridum difficile* colitis cases have risen dramatically (to 546 cases / 100,000 population) with a 2-1/2 fold rise in mortality just over 1993-2003. There is rising public awareness of antibiotic "collateral damage" but relatively little impact on demand for antibiotics.
Conclusions: 1) The great value of antibiotic "magic bullets" and vaccines has been countered by adverse effects and rising antibiotic resistance; 2) Future hope rests on: education (physician & public, elective & mandatory), disease management initiatives (practice guidelines), point-of-service behavior modification (physician computer order entry), and incorporating appropriate antibiotic use initiatives into clinical trials and marketing.

Trans-lymphatic Chemotherapy

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Background: Lymph node metastasis is a significant prognostic factor for most cancers. Failure to control lymphatic metastasis may result in local recurrence and systemic metastasis. A trans-lymphatic chemotherapy technology was developed to control lymphatic metastasis.
Methods: The technology involves an implantable gelatin sponge impregnated with biodegradable polymer microparticulate anticancer agent and is referred as microparticulate lymphatic targeting system (MLTS). When the system is formulated for paclitaxel (PTX) or doxorubicin (Dox), it is designated as MLTS-PTX or MLTS-Dox respectively. The systems were characterized *in vitro*. The pharmacokinetics (PK) of MLTS-PTX was studied in rats with comparison to injectable PTX given iv or intrapleurally (ipl). The therapeutic efficacy was examined in an orthotopic lung cancer model. MLTS-PTX was placed into the pleural cavity when the tumor lung was resected 14 days after orthotopic tumor implantation. Tumor recurrences were assessed 32 days following the procedure. The therapeutic efficacy of MLTS-Dox was examined in SCID mice bearing DLD1 orthotopic colon cancer with 100% incidence of lymphatic metastasis. Seven days after tumor implantation on the cecal wall, animals were treated with either ip implantation of MLTS-Dox, placebo sponge or no treatment. Lymphatic metastasis was examined in 40 days.
Results: Both systems exhibits controlled drug release properties *in vitro*. The microspheres were selectively taken up by the lymphatics and delivered to the regional lymph nodes as the sponge disintegrated. PK studies revealed a significantly higher AUC in mediastinal lymph nodes with ipl placement of MLTS-PTX as compared to iv or ipl administration of PTX. This represents approximately a 400-fold increase in lymphatic drug exposure as compared to iv dosing. Peak plasma concentration was significantly reduced. There was an 80% reduction in lymph node metastasis with MLTS-PTX treatment. The microparticulate PTX was microscopically evident in the targeted lymph nodes. Similarly, MLTS-Dox significantly decreased the incidence of lymph node metastasis in the treatment arm (20%) as compared to the controls (100%). Microparticulate Dox were seen in the targeted lymph nodes.
Conclusions: Trans-lymphatic targeted chemotherapy reduces lymph node metastasis in both lung cancer and colon cancer models. This effect may be attributed to the improved lymphatic distribution of the therapeutic agents.

An enzymatic approach for developing heparan sulfate-based drugs

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Background: Heparan sulfate (HS) represents a substantial portion of glycans that perform essential physiological functions. Heparin, a special form of HS, is a commonly used anticoagulant drug. The wide range of biological functions of HS attract considerable interest to exploit heparin or heparin-like molecules for the development of anticancer, antiviral drugs and a better anticoagulant drug. Heparin is currently isolated from pig intestine. A contaminant in the heparin could lead to severe allergic reactions and deaths as it happend recently in US and Germany. Thus, a cost effective method for preparing synthetic heparin is highly desirable. HS is a sulfated polysaccharide, and the unique sulfation patterns dictate the biological activity. Chemical synthesis of HS oligosaccharides that are larger than hexasaccharides is extremely difficult.
Methods: We have developed an enzyme-based approach to synthesize heparin and heparan sulfate. There are total of 13 specialized sulfotransferases and one epimerase involved in the biosynthesis of HS. We have expressed most of these enzymes in E. coli, permitting the access of a large amount of proteins. A low cost sulfo donor system was also successfully coupled with the synthesis, which reduced the cost of the synthesis by more than 1000-fold.
Results: Our method has demonstrated the feasibilities of the synthesis of the heparan sulfate with different biological functions in mutli-milligram scales. This method was employed to identify novel structures of anticoagulant HS, known as Recomparin. In addition, using structurally based mutagenesis approach, we are able to alter the susbtrate specificities of sulfotransferases. The engineered sulfotransferases allowed to synthesize those polysaccharides that can not be achieved by wild type proteins. Our results have demonstrated the potential of the enzymatic approach to prepare HS-based therapeutic agents.

Intrinsic antibiotic resistance mechanism of Mycobacteria

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Mycobacteria including Mycobacterium tuberculosis are naturally resistant to most common antibiotics and chemotherapeutic agents. The underlying molecular mechanisms are not fully understood. The mycobacterial cell wall, with its unique chemical composition and physical structure, plays a major role in the natural resistance. However, our studies of hypersusceptible mutants suggest that other mechanisms also exist. I will discuss the role of these factors in the natural resistance of mycobacteria. Understanding the molecular mechanisms of natural resistance may provide insights into the development of new generation of antimycobacterial agents or novel combinations of existing drugs.

Monitor of Chemosensitivity by Bcl-2 Transcript Kinetics in Acute Myeloid Leukemias

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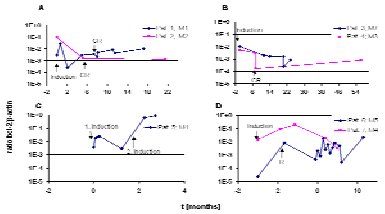
Background: Enforced bcl-2 gene expression suppresses apoptosis and confers resistance to anticancer drugs. Aims: 1) to develop the real time quantitative PCR that can monitor the expression of the bcl-2 transcript in the therapeutic course of AML patients; 2) to analyze the association between the bcl-2 gene expresssion and clinical chemosensitivity in acute myeloid leukemia.
Methods: A total of 53 patients with acute myeloid leukemia were evaluated in this study. Mononuclear cell fractions were isolated from the peripheral blood of 5 patients and classified as T-cells, B-cells, granulocytes. A) Standard curve construction: The standard curve for bcl-2 transcripts was constructed and the house-keeping gene β-actin was prepared as an endogenous reference. B) Real time quantitative TaqMan PCR was performed in the ABI Prism™ 7700 Sequence Detector System. C) Statistical analyses were performed using MS Excel 7.0 computer software.
Results: A quantitative RT-PCR assay of the bcl-2 gene, using TaqMan™ fluorogenic detection system was developed, which detected accurately the changes of the bcl-2 in the course of the chemotherapy for AML patients. The bcl-2/β-actin ratio from the patients with AML was various, but not related to FAB subtypes. This transcript ratio was not affected by mononucleated cell types. The rapid decrease of the bcl-2/β-actin ratio in samples by the real time quantitative PCR substantiated the early response, remission induction and the susceptibility to the chemotherapy protocols (Figure A & B). In the opposite, the gradual elevation of the bcl-2/β-actin ratio demonstrated the loss of effect in update-therapy protocol and the drug-resistance in AML patients (Figure C & D).



Conclusions: By applying real time PCR to clinical samples, although the bcl-2/β-actin ratio was not related to FAB subtypes, the changing data following remission induction therapy clearly reflected drug-sensitivity. These results suggest that RT-PCR assay monitored the efficacy of the chemotherapy by quantifying the bcl-2 gene transcript in AML.

Analgesic Activity of Dragon's Blood Caused by Interaction of Its Components—Cochinchinenin A, Cochinchinenin B, and Loureirin B

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Background: Dragon's blood is a renowned traditional medicine with analgesic activity. Elucidation of mechanism and material basis for its analgesic effect is important to research and development of little side-effect analgesic medicine. Aims: 1) To clarify analgesic mechanism of dragon's blood. 2) To identify its corresponding material basis. 3) To evaluate interaction between components of dragon's blood in producing analgesic effect.
Methods: Cochinchinenin A, cochinchinenin B and loureirin B were components extracted from dragon's blood. According to their percentage contents in dragon's blood, two or three component combinations were prepared. Using patch clamp technique and microelectrode extracellular recordings, effects of dragon's blood and its component combinations on voltage-gated sodium currents in dorsal root ganglion neurons and on noxious stimulation evoked discharges of wide dynamic range (WDR) neurons in spinal dorsal horn of SD rats were observed (10 neurons per drug). With pharmacodynamic parameter values of dragon's blood as reference, those of each combination was compared with the reference to identify which combination can take the place of dragon's blood. Based on concept of dose equivalence, zero interaction response surfaces used to assess interaction between three drugs with dissimilar Hill coefficients was proposed. Applying it interaction of various combinations on sodium currents and discharges of WDR neurons were evaluated.
Results: Both dragon's blood and its component combinations not only modulated tetrodotoxin-sensitive and tetrodotoxin-resistant sodium currents but also inhibited discharge frequencies of WDR neurons. Only combined effects of cochinchinenin A (0.38mmol/L), cochinchinenin B (0.19mmol/L) and loureirin B (0.08mmol/L) were similar to effects of dragon's blood (0.05%). Inhibition rates of combination and dragon's blood on discharge frequencies were (29.79±2.51)% and (30.56±2.09)%, respectively. The combined effects were defined as synergistic.
Conclusions: 1) Dragon's blood interfere not only with transmission of pain in primary sensory neurons but also with processing of pain in spinal dorsal horn. 2) Analgesic activity of dragon's blood was caused by synergistic interaction of three components—cochinchinenin A, cochinchinenin B, and loureirin B.
Authors' disclosure statement:
The experimental results that the effects of dragon's blood and its component combinations on noxious stimulation evoked discharges of wide dynamic range (WDR) neurons in spinal dorsal horn of SD rats have not been reported. Due to space limitation, only the numerical results obtained in this part have been included. In addition, our study further finds that the inhibition of dragon's blood on capsaicin (CAP)-activated currents and CAP-evoked depolarization was greater than that of cochinchinenin B. It is inferred that the above inhibition may correlate with the analgesic effect of dragon's blood and the combination of three components may have antagonistic effect on modulation capsaicin receptor similar to dragon's blood.

Matrix metalloproteinases at BBB and beyond in Multiple Sclerosis and HIV-dementia. New perspectives for therapeutical interventions

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Matrix metalloproteinases (MMPs) are extracellular zinc-dependent neutral proteinases that play an important role in physiological processes that involve tissue remodelling. Recent evidence suggests that dysregulation and imbalance between MMPs and endogenous tissue inhibitors of MMPs (TIMPs) might contribute to different pathological conditions.
Among MMPs, the subfamily of gelatinases seems to be involved in mechanisms of T cell migration into the CNS, blood-brain barrier (BBB) disruption and demyelination in the course of MS and HIV-associated neurological diseases such as AIDS Dementia Complex (ADC).
Given the importance of MMPs as key mediators in the pathogenetic mechanisms of MS and ADC, targeting MMP enzyme activity may constitute a novel therapeutic strategy in the treatment of these diseases.
We studied the role of MMPs as key mediators in the pathogenesis of MS and ADC, with particular attention to the effect of therapy on MMP secretion and expression.
By using an *in vitro* model we investigated whether IFN-β as well as the antiretroviral drugs zidovudine (AZT) an indinavir (IDV), drugs used for the treatment of MS and HIV-infected patients, respectively, are able to modulate the activity and the expression of MMPs in glial cell cultures.
As assessed by gelatine-zymography and RT-PCR, we observed a dose-dependent inhibition of MMP-9 activity and expression in both LPS-activated astrocytes and microglia. MMP-2 inhibition by IFN-β and antiretroviral drugs was observed only in astrocytes but not in microglia.
On the bases of these *in vitro* results we also investigated the effect of antiretroviral therapy on the release and the expression of MMP-9 from circulating peripheral blood mononuclear cells (PBMC) from HIV-infected individuals. By using a sensitive fluorescent-activated substrate conversion (FASC) assay we demonstrated the presence of active MMP-9 in PBMC supernatants from HIV-infected patients naïve for antiretroviral therapy (ARV). By contrast, in both healthy donors and ARV-treated subjects, there was no MMP-9 net activity, indicating that MMP-9 was completely blocked by binding to its natural tissue inhibitor TIMP-1.
These results outline the possibility to use antiretroviral drugs and compounds with anti-inflammatory properties, which have been shown to inhibit MMP function, for the experimental treatment of neurological disorders in which the inhibition of MMP could have clinical benefits.

Inhibition of HIV-1 through an Innate Humoral Mechanism – a Potential for Vaccine Development

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Background: In prior studies we show that naturally occurring IgM anti-leucocyte autoantibodies (IgM-ALA) bind to certain leucocyte receptors e.g. CD3, CD4, CCR5 and CXCR4 but not to others e.g. CD8, CD28 and HLA. IgM-ALA inhibit T cell function, chemokine binding to receptors and chemotaxis of leucocytes. Using monoclonal IgM, derived form umbilical cord B cell clones, we show that <10% clones have IgM-ALA activity and <1% clones bind to CD4 indicating that binding of IgM to receptors is highly specific. Umbilical cord sera contained IgM-ALA but not IgG-ALA. We wanted to determine if IgM-ALA by binding to co-receptors, inhibits HIV-1 entry into cells.
Methods: IgM and IgG was purified from 10 normal and 25 HIV sera using size exclusion column chromatography and contaminating IgG from purified IgM was absorbed out.
Results: Physiological doses of purified individual IgM, but not IgG and not IgM pre-absorbed with leucocytes, from normal and HIV-1 sera inhibited (>95%) both X4 and R5 HIV-1 from infecting PHA+IL2 activated human PBL in-vitro and in vivo in human PBL-SCID mice. HIV-1 infectivity of PBL was also inhibited with human monoclonal IgM (2 clones) having anti-CD4 activity but not with IgM (6 clones) that lacked binding to leucocytes indicating that IgM mediated inhibition of HIV-1 is highly specific. We show that IgM inhibits HIV-1 attachment to core receptors as IgM inhibited syncytia formation (by >90%) and inhibited infection of R5 and X4 pseudo typed virus. IgM from certain individual HIV-1 sera were not inhibitory to some R5-HIV-1 viral strains and did not bind to CCR5 receptors indicating that certain HIV-IgM may lack antibodies reactive to strain specific co-receptor epitopes.
Conclusion: An innate humoral mechanism which is present from birth i.e. IgM-ALA, has a role in inhibiting HIV-1 viral entry into cells. Developing strategies to enhance in-vivo IgM-ALA e.g. through a vaccine, could prolong the asymptomatic state in HIV-1 infected individuals. This work has been published (J of Immunol, 2008. 180: 1769).

Multiple antibiotic resistance of heterotrophic bacteria from Siberian lakes as an indicator of anthropogenic influence on the ecosystems

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Background: Antibiotic usage in medicine practice and veterinary has led to dissimination of genes multiple antibiotic resitstance (MAR) among natural aquatic bacteria. Aimes: To examine the effect of anthropogenic impact on appearance of MAR by heterotrophic bacteria isolated from two lakes differing in anthropogenic impact level and bacterial plasmid profile.
Methods: Heterotrophic bacteria isolated from central and resort/shore-line parts of Lake Shira/Lake Shunet (Southern Siberia) were investigated. The method of replica plating was used for antibiotic sensitivity testing. The following antibiotics were used: amikacin (30 µg/ml), ampicilin (50 µg/ml), benzylpenicillin (10 µg/ml), cefotaxime (25 µg/ml), doxycycline (30 µg/ml), gentamicin (10 µg/ml), kanamycin (30 µg/ml), streptomycin (30 µg/ml). Isolation of plasmids was carried out by the method of alkaline lysis.
Results: The antibiotic resistance profiles of heterotrophic bacterial isolates recovered from two lakes were determined. Resistance was detected in at least one strain for seven of the eight antibiotics tested, the exception being amikacin. No bacteria with single antibiotic resistance were found. Resistance was more frequently observed among isolates recovered from within the proximity to a tourist resort (Lake Shira), or the shore-line (Lake Shunet) than comparative samples from the centre of each lake. Bacteria with multiple antibiotic resistance (resistant to 2 and more antibiotics) were checked on a presence plasmid in their cells. We found plasmids of varying both in sizes (from less than 2.3 to <23.1 kb) and their number (from 1 to four per cell). Plasmids of medium size (about 23.1) predominated in bacterial cells. The same plasmid profile was found for bacteria isolated from the central and from the resort parts of Lake Shira and these bacteria have the same antibiotic resistance pattern.
Conclusions: Multiple antibiotic resistance of heterotrophic bacteria studied is due to anthropogenic impact on the ecosystem and this property of bacteria can be used for ecological monitoring on the lakes. One of the possible reasons of the plasmid distribution among bacteria may be plasmid capture by bacteria under human activity conditions.

Specificity of multiple antibiotic resistance appearance by luminous bacteria inhabiting the Pacific and Indian oceans

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Background: A level of pollution of World ocean by bacteria containing R-plasmids with genes encoding for multiple resistance to antibiotics (MAR) is determined by both density of population of seasides and presence on them cities and resorts. Reistance of luminous bacteria to high concentrations of antibiotics can be used as a marker of anthropogenic influence on sea ecosystems. Aimes: To study resistance of luminous bacteria to different antibiotics and to evaluate possible mechanisms of bacterial MAR.
Methods: Luminous bacteria (180 strains) belonging to the *Photobacterium leiognathi*, *Photobacterium phosphoreum* and *Vibrio harveyi* isolated from water and different sea inhabitatnts of the Pacific and Indian oceans were investigated. The method of replica plating was used for antibiotic sensitivity testing. Mechanisms of bacterial antibiotic resistance were investigated on molecular, cellular and population levels.
Results: About 90% of all strains studied appeared resistance to high concentrations (50-500 mkg/ml) of ampicillin and penicillin. Resistance of the strains to kanamycin and streptomycin ranged from 30 till 90%, to rifampicine, tetracycline and to doxycycline was no more than 50% and 15% correspondingly. Bacteria isolated from Indian Ocean were found to be resistant to doxycycline, and resistance of strains from the Pacific Ocean to this antibiotic was not exceeding 5%. Strains of *P.phosphoreum* were more sensitive to used antibiotics than other strains under the study. Strains of *V.harveyi* isolated from both oceans appeared the highest level of MAR. Strains of *P.leiognathi* isolated from Indian Ocean were resistant to 4-8 antibiotics while in the Pacific Oceans the same bacteria were resistant to 2-4 antibiotics.
Conclusions: The largest number of bacterial strains with MAR was found among free living of *V. harveyi* isolated from coastal zones of both oceans. The tendency to increasing of MAR was found of *P.phosphoreum, P. leiognathi, V. harveyi.* In the Pacific Ocean bacteria with MAR were isolated mainly from coastal zone, and in Indian Ocean through all water basin. Bacteria with high level of MAR were chosen as potential plasmid-bearing strains.

Identification of the cocaine binding site on the dopamine transporter

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Background: Cocaine is a widely abused substance with psychostimulant effects attributed to inhibition of the dopamine transporter (DAT). Currently, there is no medical treatment against cocaine addiction. Interestingly, analogues of benztropine (BZTs) are less effective than cocaine as behavioral stimulants, despite having similar or higher affinity and selectivity for the DAT than cocaine. In fact, some BZTs have shown to antagonize the effect of cocaine in rea behavioural models.
Methods: We use molecular docking models dopamine, cocaine and BZTs to assess the binding site of the compounds in the DAT. The models were validated experimentally using systematic mutagenesis of the involved residues and studyint the effect on substrate affinity, We also assessed the correctness of the docking model of the cocaine analogue, CFT, by the engineering of a zinc binding site and using chemical cross-linkers.
Results: The models of dopamine, CFT and BZTs show almost complete overlap with only a few residues shown to be unique for either compound. Systematic mutagenesis of the residues proposed to be involved completely validated the models. Trapping of the radiolabeled cocaine analog [3H]CFT in the transporter, either by cross-linking engineered cysteines or with an engineered Zn2+-binding site that was situated extracellularly to the predicted common binding pocket also produced results in agreement of the docking model. In particular, the orientation of Tyr156 in TM3 showed marked differences between the models: In the model for dopamine and BZTs, a hydrogen bond is formed between the OH-group of Tyr156 and Asp79 in TM1. In contrast, docking of CFT causes a disruption of this H-bond. Disruption of this interaction (Y156F) resulted in several fold decrease in affinity for dopamine and BZTs but had no effect on the affinity for CFT.
Conclusions: Our data show the molecular basis for the competitive action of cocaine at DAT. We also demonstrate in DAT a unique binding mode for cocaine, which unlike substrates and BZTs, produce a conformational rearrangement of the binding site that disrupts a stabilizing OH-bond between Tyr156 in TM3 and Asp79 in TM1.

Antisecretory Factor (AF) - an inducible antisecretory and antiinflammatory protein

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Background. The Antisecretory Factor (AF) is a 41 kda protein which affects ion/water transport and inflammation in the small intestine.The endogenous plasma level of AF is increased by enterotoxins and by certain food constituents such as hydrothermally processed cereals. The active site of AF is a 16-peptide (AF16) at position 36-51. We here show that the peptide also exerts effects on the central nerve system.
Methods. Intracranial pressure was measured in rats with a fiber optic transducer positioned at the posterior parts of the lateral ventricle in the brains. An elevated pressure was induced by infection with Herpex simplex virus causing encephalitis (HSE); 25 μg of AF16 (n=10) or vehicle alone (n=22) was administrated intranasally twice daily. In order to measure in vitro effects of AF16 nerve cell membranes from Deiters cells were mounted in microchambers and $36Cl^-$ permeability studied.
Results AF16 rescued all rats with HSE; in contrast 90 % of animals given vehicle alone died. The effect of AF16 was probably due to its capacity to reduce intracranial pressure since a single dose of the peptide reduced the pressure to normal. In vitro pmol levels of AF16 was shown to counteract the out to in $36Cl^-$ permeability in nerve cell membranes; in contrast AF peptides lacking the active sequence had no effect.
Conclusion The results suggest that AF affects ion/water transport not only in the gut but also in the central nerve system illustrating once more the so called gut/brain axis.

Inintracavitary-administered Nimotuzumab labeled with 188Re in adult recurrent high-grade glioma.

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Radioimmunotherapy (RIT) may improve the management of malignant gliomas. Nimotuzumab is a humanized monoclonal antibody directed against epidermal growth factor receptors. Three patients with anaplastic astrocytoma (AA) and 8 with glioblastoma multiforme (GBM) were intended to be treated with 3 mg of mAb labelled with 10 or 15 mCi of 188Re. In patients treated with 10 mCi (n = 6) transitory worsening of pre-existing neurological symptoms were observed. Two patients treated with 15 mCi (n = 4) developed early severe neurological symptoms and one also developed late severe toxicity (radionecrosis). In the group treated with 10 mCi, 1 GBM patient died in progression 6 months after the treatment, 2 patients (1 GBM and 1 AA) developed stable disease during 3 months. One GBM patient had partial response for more than 1 year and 2 patients (1 GBM and 1 AA) were asymptomatic and in complete response after 3 years of treatment. Maximal tolerated dose of the radioimmuno-conjugate 188Re-Nimotuzumab was 3 mg of the h-R3 labelled with 10 mCi of 188Re.The radioimmuno-conjugate showed a high retention in the surgical created resection cavity and the brain adjacent tissues with a mean value of 85.5% of the injected dose one hour post-administration. This radioimmunoconjugate may be relatively safe and a promising therapeutic approach for treating high grade gliomas.

Virostatics: a new class of immunomodulators with dual antiviral and cytostatic properties to inhibit viruses and protect the immune system from hyperactivation during chronic infections

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Background: Chronic infections are characterized by continued stimulation of the immune system, often resulting in variable degree of hyperactivation with consequent partial or complete immune exhaustion, as it is the case for HIV/AIDS.
Methods: We have characterized drugs or combination of drugs carrying both antiviral and cytostatic properties, and named them virostatic drugs. In particular, we have analyzed anti-HIV compounds, and screened them for antiviral and antiproliferartive properties both in activated and quiescent lymphocytes. VS411, a fixed combination of two drugs, is the leading compound.
Results: VS411 has successfully completed a bioavailabilty Phase I study. Formulation has been chosen and VS411 is presently in Phase II development program. Previous academic Phase II studies have shown that VS411 is effective at reducing viral load in HIV infected individuals. A second generation of virostatic drugs is undergoing a preclinical program in our laboratories.
Conclusions: Virostatic drugs represent a new family of antivirals designed not only to suppress viruses but also to preserve the immune system from chronic damage. VS411 is is a fixed combination of virostatic drugs undergoing Phase II clinical studies that appears to be safe, well tolerated, and effective at reducing viral load.

AdCD40L Cancer Vaccine – From Experimental Models to Clinical Application

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Background: Cancer vaccines are merging as novel treatment options for cancer patients. The first line of vaccines encountered obstacles such as tumor immune escape. Novel strategies have been explored that aim to both activate anti-tumor immunity as well as hamper the regulatory mechanisms exhibited by the tumor. The objective for our tumor vaccine program was to evaluate the efficacy of AdCD40L therapeutic vaccination for solid tumors such as bladder cancer.
Methods: Adenoviral vectors were used to transfer the immunostimulatory gene CD40L into tumors. Preclinical evaluation of tumor eradication and development of anti-tumor immunity was made in our murine bladder cancer model as well as in both murine and human culture systems. Clinical evaluation was made in patients with bladder cancer in a clinical phase I/IIa trial. Tumor immunity upon therapeutic vaccination was investigated with techniques such as CBA, quantitative PCR, proliferation assays, and flow cytometry.
Results: In a series of publications we have demonstrated that AdCD40L can cure highly aggressive bladder cancer in experimental models. In these models, AdCD40L was shown to efficiently activate tumor-specific immunity by maturing dendritic cells, stimulating Th1 cytokines and activating cytotoxic T cells. Further, the levels of T regulatory cells and suppressive cytokines such as IL10 and TGFb were decreased which may be crucial for the anti-tumor effect. In recently obtained unpublished animal data, local AdCD40L administration into tumor-positive bladders eradicated both bladder tumors and distant lung metastases. Currently, a clinical evaluation is performed. Phase I is completed (n=5) and AdCD40L vaccination was safe. No side effects have been documented. Routinely, bladders are removed from patients with high-grade malignancy to avoid incurable metastases. After AdCD40L therapy no high-grade tumor cells could be detected in the cystectomized bladders. Further, T regulatory cells were reduced after treatment in compliance to our experimental models.
Conclusion: AdCD40L cancer vaccination seems to eradicate high-grade malignant tumor cells and is a promising candidate for therapeutic vaccination of both local and disseminated malignancy.

Effects of Theranekron (alcoholic extract of Tarantula cubensis) in treatment of Foot- and- mouth disease (FMD) lesions in cattle

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Background: Foot- and- Mouth disease (FMD) is an acute viral disease of all cloven- footed animals. In enzootic countries where a slaughter policy is not in force, treatment of infected animals is recommended. Theranekron (Richterpharma, Austria) contains the whole extract from spider tarantula cubensis.In the present study therapeutic effect of Theranekron on foot- and-mouth disease in cattle was investigated.
Methods: During an outbreak of FMD in cattle in Iran, fifty infected cattle (in early stage of disease) treated with single subcutaneous injection of Theranekron (treatment group). Fifteen infected animals at the same time chose and treated as follow: daily injection of an anti inflammatory drug, broad spectrum antibiotic and daily dressing of lesions with a mild disinfectant for three to five days. Clinical examination and observation of lesions in infected animals (treatment and control groups) was carried out in 24, 48 and 72 hours and also one and two weeks after treatment.
Results: Rectal temperature (fever) in Theranekron injected group subsided to normal range after 24 hours of injection, and mean values of rectal temperature in treatment group during study was significantly lower than animals in control group ($p< 0.05$). in table the results of treatment in two groups on oral mucosal lesions of infected cattle has been appeared.
A significant difference between median value of oral lesions in treatment and control
groups were showed in 24 hours and days 2, 3 and 7 after treatment by Mann-whitney test ($P<0.05$).

Day / Group	Before Treatment	24 hours next	2 days next	7 days next	14 days next	NO
Control	1 (1- 1)	1 (1- 2)	2 (2- 1)	3 (4- 3)	4 (4- 4)	15
Treatment	1 (1- 1)	2 (5- 2)	3 (4- 3)	4 (4- 4)	4 (4- 4)	50

1) Blister , 2) Burst blister with hyperemia , 3) Reepithelialization of lesions
4) Complete healing, 5)Reabsorption of vesicles without bursting
Appetite in treatment group had been returned to normal after 48 hours and in control group lasted for 4 days.
Conclusion: From results of present study it appears that Theranekron is a very efficient drug for treatment of FMD cases in cattle especially because of its anti inflammatory and healing properties and convenient in use (single injection) compare to the routine treatment.

Cardioprotective Anthracycline PKC Activators for The Treatment of Drug-Resistant Tumors

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Successful cancer chemotherapy is often limited by cellular drug resistance, which requires dose escalation for circumvention, and by systemic adverse effects, which limit the cumulative dose of drug administered. Typical of these dual limitations are the anthracycline antibiotics, such doxorubicin (DOX), whose cytotoxicity is blocked by multiple resistance mechanisms, including the expression of multidrug transport proteins (MRP-1, P-gp), anti-apoptotic proteins (Bcl-2, Bcl-XL), proliferative proteins (NF-kB, Bcr-Abl kinase), and by p53 protein dysfunction. DOX efficacy is also limited by well-characterized and often irreversible cumulative cardiotoxicities linked to the generation of reactive oxygen species (ROS) by the anthraquinone ring.
In response to these therapeutic impediments, we have developed a novel class of anthracyclines, represented by *N*-benzyladriamycin-14-valerate (AD 198) and *N*-benzyladriamycin-14-pivalate (AD 445). AD 198 and AD 445 are markedly more lipophilic than DOX and, consequently, circumvent efflux by multidrug transport proteins. Unlike DOX, AD 198 and AD 445 localize in the cytoplasm and do not target DNA but, rather, bind to the C1b (diacylglycerol-binding) domain of protein kinase C (PKC). Drug-mediated PKC-delta activation triggers rapid apoptosis in proliferating cells through a novel mitochondrial-dependent pathway and in a manner that circumvents the anti-apoptotic effects of Bcl-2 and Bcl-XL expression. Since apoptosis is triggered rapidly and without the requirement of cell cycle arrest, enhanced proliferative signaling or p53 dysfunction do not block AD 198/AD 445-mediated apoptosis.
Despite the retention of an anthraquinone ring, AD 198 is non-cardiotoxic in the Bertazzoli (chronically-dosed) mouse model. This correlates with the activation of PKC-epsilon by AD 198 and enhanced cardioprotective signaling in cardiomyocytes, which protects the heart from reperfusion injury following induced ischemic or from high-dose DOX-induced injury in an *ex vivo* perfused heart model. Our studies suggest that AD 198 and AD 445 may provide improved therapy for drug-resistant tumors without concern for dose-limiting cardiotoxicities associated with conventional anthracyclines or with cardioprotection when administered in combination with potentially cardiotoxic antitumor agents.

Proposed antiangiogenic agents with mechanisms that prevent tumor cell survival and resistance to cytotoxic therapies

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Background: The transcription factor hypoxia inducible factor-1α (HIF-1α) facilitates cell survival and angiogenesis through transcriptional activation of multiple target genes including vascular endothelial growth factor (VEGF). HIF-1α has also been linked to increased drug resistance through expression of the ABCB1 drug transporter, preventing intracellular drug accumulation. We recently found that loss of methylation-controlled J protein (MCJ) correlates with increased expression of ABCB1 and increased resistance. Others have also shown that inhibition of glycogen synthase kinase 3β (GSK3β) promotes drug resistance and that HIF-1α contains consensus sites for phosphorylation by GSK3β. Because MCJ regulates protein translation/degradation and HIF-1α is primarily regulated by proteasomal degradation, we tested the hypothesis that HIF-1α is negatively regulated by MCJ through sites phosphorylated by GSK3β.
Methods: This study used MCF-7 breast cancer cells, SKOV-3 ovarian cancer cells and HEK 293 cells. ShRNA expression constructs were used to reduce MCJ, and overexpression of GSK3β was accomplished by constitutively active GSK3S9A. HIF-1α levels were determined by Western blot, transcription was measured by quantitative PCR, and protein interactions were identified by co-immunoprecipitation.
Results: Inhibition of MCJ expression or GSK3β activity resulted in increased HIF-1α expression, VEGF transcription and drug resistance. Conversely, overexpression of constitutively active GSK3β led to both a decrease in hypoxia-driven HIF-1α protein levels and VEGF transcription. Interaction between GSK3β and HIF-1α was detected by co-immunoprecipitation. Mutation of the putative GSK3β phosphorylation sites within HIF-1α promoted an increase in protein level and a decrease in ubiquitination of the HIF-1α mutant protein.
Conclusions: This study illustrates a mechanism in which posttranslational modification by GSK3β attenuates HIF-1α possibly through MCJ interaction, ubiquitination and proteasomal degradation, thus decreasing angiogenic potential and preventing drug resistance. We propose the future study of GSK3β activation by means of PI3 kinase inhibitors as therapy to prevent angiogenesis and drug resistance in cancer cells.

A Potential Anti-Cancer Drug From a Plant Extract, *Tillandsia recurvata*

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Background: Most successful cancer drugs have been derived either directly or indirectly from plant materials. Jamaica is fortunate to have 84 of the 120 recognized medicinal plants of the world. One of these plants, *Tillandsia recurvata* has demonstrated potent anti-cancer activities both invitro and invivo. The following specific activities were done: 1) Isolation and purification of bioactive compound from this plant extract; 2) The anti-cancer properties were tested invitro and invivo activities of this isolated compound; and 3) Assessed through preliminary findings one of the mechanisim of action of this compound
Methods: *Tillandsia recurvata* was collected in Jamaica. The fresh plant material was dried in steam oven at 70°C then macerated and then extracted with cold MeOH. Various fractions were then isolated be colume comotography. Subfractions were then processed and tested via bioguided fractionation. This was follwed by using advanced separation technology (HPLC, supercritical fluied chromatography and capillary electropheresis. This was followed by LC/MS and finally NMR). This study involves the testing of the isolated fractions against 5 different histogenic tumors invitro using the trypan blue exclusion invitro assays. We also utilized the 3H Thymadine incorporation assay. The five tumor cell lines used in these assays were the following: 1) melanoma; 2) prostate; 3) breast; 4) Kaposi sarcoma and 5) b-cell lymphoma. The extract was tested invivo against the above tumor cell lines. The invivo studies included 10 mice per group and using the crude form of the extract at 10mg per mice per day were for 7 days. This was done for each of the above tumor cell lines compared with controls with normal saline treatment. The purified compound is now currently being produced at ground levels to test invivo.
Results: Utilizing the bioassay-guided fractionation process, the bioactive moiety was isolated at 98% purity. This purified compound was tested invitro and demonstrated to be higly effective at a rate of 95% to 100% cell kill in the invitro assays of 5 different histogenic tumor cell lines. The invivo studies were equally as impressive utilizing the crude extract. All tumors responded to the treatment by reducing the tumor size from .4mm X .4mm to almost non existent on gross examinations of all the above tumor cell lines. On histology 90% to 95% of the tumors were undergoing cell death. Using immunohistochemical staining we were able to determine that the cell death was due primarily to induced apoptosis. No toxic signs or systems were observed in any of the invivo studies.
Conclusions: This newly extracted compound demonstrated a significant anti-cancer properties. Preliminary studies from this newly isolated compound indicates that this compound may serve as an excellent new anti-cancer drug.

Authors' disclosure statement This compound has been patented.

A New Molecular Mechanism of Action of a Leading Chemotherapeutic Drug—Cisplatin and Its Novel Applications

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Background: There is pressing need for mechanistic understanding of action of existing anticancer drugs at the molecular level, which can, in turn, lead to molecular-mechanism-based design of new anticancer drugs. Despite its great success in treating certain cancers, cisplatin as the most effective chemotherapeutic drug has severe toxic side effects and both intrinsic and acquired resistance. Such drawbacks have even prompted the call to discontinue the therapeutic applications of cisplatin-like anticancer drugs. One outstanding problem is that the precise mechanisms of action of these anticancer drugs remain elusive. Aims: 1) To obtain a true understanding of the molecular mechanism of action of cisplatin. 2) To optimize cisplatin chemotherapy. 3) To apply our mechanistic understanding of cisplatin to developing new effective cancer therapies, including combinations with radiotherapy and photodynamic therapy.
Methods: *Time-resolved ultrafast (femtosecond) laser spectroscopy* is the most powerful technique for real-time observations of molecular reactions since it uses laser flashes of such short duration down to the time scale on which the reactions actually happen – femtoseconds (fs) (1fs= 10^{-15} second). The molecular mechanism of anticancer drugs revealed by this technique is examined with biomedical methods such as DNA damage and cell death measurements.
Results: An extremely high reactivity of cisplatin with electrons and a new electron transfer mechanism of the cisplatin-DNA interaction have been discovered. Furthermore, we have revealed the molecular reaction mechanism of the combination therapies of low-dose cisplatin with radiotherapy and photodynamic therapy. Based on our mechanistic understanding of cisplatin, new molecular regulators have been developed to enhance the therapeutic effects and to reduce the side effects.
Conclusions: 1) The therapeutic effectiveness of cisplatin is closely related to its high reactivity with electrons. 2) This finding can be utilized to improve the chemotherapy with cisplatin and to develop new combination therapies for effective treatment of cancers.
References: 1. Q.-B. Lu et al., *Molecular Pharmaceutics* **4**, 624(2007). 2. Q.-B. Lu, *Journal of Medicinal Chemistry* **50**, 2601(2007).

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MHC Molecules as Antigen Receptors?

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The speculation that immunologically reactive haptens must be those attached to carriers' immunodominant epitopes immediately suggests a clearer mechanism by which the mysterious hapten-carrier phenomena are generated. In a specified T-B cell cooperating response against an antigenic determinant, T and B lymphocytes specifically recognize this specified determinant of the same antigen molecule in two different ways and in different circumstances. The B cell recognizes an antigen by the preliminary antigen receptors on the cell's surface, at the time it is still intact. In contrast, the T cell recognizes the hidden structure of this epitope in specific amino acid sequences that are first recognized by MHC class I and II molecules on the surface of APCs. This analysis revealed that only a small number of regions, called epitopes or determinants, in a protein antigen are immunogenic and capable of stimulating humoral and cellular immune responses. The program of immune response against an antigen must be similar to the program of fertilization, resulting in one cell, one antibody. Since the transfer of tissue between individuals is rare in Nature, graft rejection cannot be the primary function of MHC proteins. The concept that immunoglobulins on B cell membrane are antigen receptors has been challenged and may be replaced by the new hypothesis that all antigen presenting cells use their MHC class II molecules as receptors for antigen recognition to initiate humoral immune response. This is in view of the fact that no MHC II molecule has yet been found in T cells. This hypothesis supplies a single explanation for the anamnestic immune response. Alternatively, antigen presenting and processing is necessary for the anamnestic response. Furthermore, it must be MHC molecule that acts as a specific "enzyme-like molecule" by unknown mechanisms with the aid of some cofactors inside the antigen presenting cells, and protects antigen from complete degradation and allows them to emerge as peptides. In developing effective vaccines against hypervariable viruses, and effective drugs for immunosuppressive therapy for transplantation, there is still a lot to learn from the hapten-carrier phenomena.

TGF-β: A primary tumor suppressor gene involved in leukemogenesis

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Background: Tumorigenesis has been show to be a multistep process. Initial genetic mutations are often associated with dysfunctional growth regulation and are often followed by alterations in tumor suppressor gene function allowing unchecked cell cycle progression, and by genomic instability, additional genetic mutations responsible for tumor metastasis. Aim: To study the importance of TGF-β, a critical immune suppressive cytokine, on T lymphocyte cell growth.
Methods: This study included 150 mixed gender transgenic (Tg) mice, which overexpress a dominant negative TGF-β II receptor (DNRII) using a human CD2 promoter to target T lymphocytes. Forty-eight homozygous, sixty-five heterozygous DNRII Tg mice and twenty control mice were monitored by periodic differential blood counts, which were used to determine disease state. Five homozygous and five heterozygous mice were selected for chromosome analysis studies and 3 additional mice were used for adoptive transfer studies.
Results: DNRII Tg mice developed a CD8 lymphoproliferative disease marked by increased expression of CD44 and IL-2Rβ. Homozygous mice developed disease, as determined by high white blood counts (WBC) more rapidly than heterozygous mice (37 vs 53 weeks). Lymphomas exhibited multiple chromosomal abnormalities, as determined by spectral karyotype analysis, including aneuploidy, deletions, and translocations as is shown in the table. Several lymphomas were adoptively transferred to irradiated recipients and shown to be capable of transferring the tumor.

ID#	Alleles	Aberration
2020	1	t[a](1;14); t(11;15) [6][b]
60	1	42,XXY [8]; 40,XY [2]; t(4;13 or 5)[4]; +5[8][c] [10]
5787	2	40,XY; t(4;5) [5]; t(14;3) [4] [12]
5664	2	38-40,XX [3]; X -X[2][†]; -8[2] [5]

[a] Translocation
[b] Number of chromosomes with aberration/total studied at end of line
[C] Addition (+) or deletion (-) of chromosome

Conclusions: Dysregulation of the TGF-β pathway in T cells results in abnormal growth in the CD8 T cell subset resulting in a lymphoproliferative disease followed by leukemia/lymphomas as demonstrated by WBC, chromosomal abnormalities and ability to transfer to syngeneic host, suggesting that TGF-β is acting as a primary tumor suppressor gene in CD8 T lymphocytes.

The gut, the forgotten metabolic organ: the story of testosterone and dextromethorphan.

LUEDTKE D

The liver is known as the major site of first pass extraction. Although a lot of efforts have been done to characterize the metabolic behavior of the intestine, the contribution of this organ to first pass extraction is often ignored. Recent studies have indicated that the small intestine contributes significantly to the first pass metabolism of many drugs in human, e.g. cylosporine, nifedipine, midazolam, diltiazem and verapamil.
Therefore, we have investigated the profile of CYP P450 proteins via Western blot analysis along the entire length of the intestine in the rat. We identified CYP3A and CYP2B1 as the major expressed CYP P450 isoforms in the rat upper intestine, while CYP2C6 and CYP2D1 dominate the lower parts of the intestine (ileum/colon). Furthermore, the expression data were compared with functional data using two well known CYP P450 substrates testosterone and dextromethorphan. Testosterone is predominantly metabolized by CYP3A and CYP2B1, while dextromethorphan is a CYP3A and CYP2D1 substrate. The data show how the intestinal metabolism might affect/limit absorption and, hence, how relevant the intestinal metabolism might be for the first pass extraction of orally administered drugs.

Specific Inhibitors of the Cyclic Nucleotide Phosphodiesterases PDE2 and PDE4 Overcome *In Vitro* and *In Vivo* Angiogenesis

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Background: Cyclic nucleotide phosphodiesterases (PDEs) play a key role, downstream receptor activation, in intracellular signalling by selectively hydrolyzing cyclic nucleotides that serve as second messengers in a number of cellular pathways. Eleven PDE isozyme families (> 100 proteins) have been characterized and are differentiated by their substrate specificity, their tissue, cell and subcellular distributions, and also by their short-term and long-term regulations. Altogether, the complexity of these famillies allows a fine and compartmentalized regulation of cyclic nucleotide levels. Among these famillies, PDE2, which hydrolyzes both cAMP and cGMP and whose the cAMP hydrolysis is stimulated by cGMP, and PDE4, which specifically hydrolyses cAMP, represent the main isozymes in human umbilical vein endothelial cells (HUVECs). Angiogenesis is defined as the formation of new blood vessels from pre-existing ones. Since angiogenesis plays a major role in tumor development induced by tumoral vascular endothelial growth factor (VEGF) secretion, nowadays anti-angiogenic efficient therapeutical approaches are developped mainly at the receptor level.
Methods: Herein, by using an *in vitro* angiogenesis model (HUVECs), an *in vivo* angiogenesis model (chicken embryo chorioallantoic membrane; CAM), and an *in vivo* tumorisation model (tumor growth induced by BF16/10 cells in 20 C57BL/6N mice), we show that the combination of PDE2 (EHNA) and PDE4 (RP73401) inhibitors overcome angiogenesis.
Results: Our studies show that VEGF-induced HUVECs proliferation and migration is associated with PDE2 and PDE4 upregulations (mRNA, proteins and activities) and that PDE2 and PDE4 inhibition increases cAMP level, inhibits cell migration and proliferation and also inhibits VEGF-induced cell cycle progression at the level of ERK phosphorylation, cyclin D1 expression. Similar studies performed with delphinidin (a grape polyphenol which inhibits PDE2 and PDE4), show that delphinidin inhibits *in vitro* (HUVECs) and *in vivo* (CAM) angiogenesis. Tumor growth treated with EHNA+RP73401 is reduced by 30% (P=0.014).
Conclusions: It clearly appears that targetting VEGF-upregulated endothelial PDE2 and PDE4 is a new and original strategy to overcome the intracellular signaling dysfunction induced by VEGF stimulation which should induce less side effects.

Prevention of Inflammatory Leukocyte Adhesions for Treatments in Acute Sepsis Condition by Anti-CD18 sFv Single-Chain Antibody

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Background: Leukocyte integrin (CD11/CD18 heterodimer) plays a major role in immune cell trafficking. Translocation of leukocytes from blood circulation to extracellular space in injured or infected tissues is essential to immune surveillance and defense against invading pathogens. However, in certain pathological conditions like systemic inflammatory response syndrome (SIRS) and bacterial sepsis, excessive infiltrated leukocytes could cause multiple organ failures. Aims: (i) to generate anti-CD18 scFv agent that could block leukocyte adhesion to injured wounds and (ii) to evaluate its efficacy on preventing inflammatory leukocyte migration in rodent sepsis model.
Methods: A phage display library was generated from splenocytes of BALB/c mice hyperimmunized with human CD18 βA antigen. Phage scFv antibodies were screened for binding activity against the CD18 antigen, and the clone was isolated and selected by three rounds of panning exercises. Antigenic specificity of the anti-CD18 scFv was determined by flow cytometry, immunohistochemistry, Western blot, and immunoprecipitation. This study also assessed the therapeutic efficacy of the purified anti-CD18 scFv (0.8 mg/kg, i.p.) compared with placebo control (each group, n = 5) in murine cecal-ligation puncture (CLP) model. We also determined the animal survival, leukocyte infiltration into liver and lung organs.
Results: A high affinity of anti-CD18 scFv phage was isolated, and subsequently produced in large quantity in E. coli. The purified scFv agent was shown to bind specifically to the CD18 antigen as shown by immunoprecipitation and Western blot. The scFv also stained in FACS analysis the Jurkat T lymphocytes, but not the Jurkat mutant lacking the CD18. In the CLP animal model, one bolus of anti-CD18 scFv, but not the placebo, could significantly reduce the severity of leukocyte infiltration into lungs and livers in the treated animals, as revealed by immunohistochemical staining of CD45+ cells. Furthermore, the scFv-treated animals revealed significantly low levels of circulating TNF-α and IL-6 pro-inflammatory cytokines. Of importance, the anti-CD18 scFv could demonstrate survival advantage in the CLP-inflicted mice.
Conclusions: 1) The scFv agent is shown to bind CD18 antigen and the eptiope mapped to the betaA domain. 2) Anti-CD18 scFv significantly blocks leukocyte infilitration into major organs (liver, lung) and attenuates the release of proinflammatory cytolines (TNF-α, IL-6) in systemic sepsis conditions. 3) Animals treated with the scFv could survive longer compared with those from the placebo group in the CLP model. Collectively, our findings suggest that blocking the CD18 βA domain by anti-CD18 scFv promises to be an effective approach for therapeutic intervention of leukocyte-mediated tissue damage.

Authors' disclosure statement: This study is supported by the Research Grants Council of Hong Kong and has been filed for US Patent (No.: 61/080,558).

according to registration: Lukawska M

Amidinoanthracyclines – Perspectives to Promising Modification of Known Anticancer Drugs

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Background: Anthracylines such as daunorubicin, doxorubicin and epidoxorubicin are commonly used antitumor drugs with wide spectrum of activity in human cancer. However, their clinical effectiveness is limited by several important factors, including dose-dependent toxicity and cardiotoxicity. Moreover the resistance of tumor cells to this drug remains also one of the major clinical problems. The attempts reported in the literature to solve these problems are based mainly on the modifications of the structure of these antibiotics by introduction of various substituents at different sites of their molecules.
In the course of our study on the properties of anthracyclines and their derivatives we have set the hypothesis, that the one of the main factors responsible for their toxicity is the presence of the primary amino group ($–NH_2$) at the position 4' of the daunosamine moiety and that its transformation into the trisubstituted amidino group (–N=CR'–NR''R'') may cause two effects: the decrease of toxicity of anthracyclines and additionally the increase of their antiproliferative activity.
Methods: To check this hypothesis 40 **amidinoanthracyclines**, were synthesized. To throw some light on the structure-activity relations the compounds in this novel family of the anthracyclines are divided into 4 analogous series, namely derivatives of daunorubicin, doxorubicin, epidaunorubicin and epidoxorubicin containing the same set of 10 amidino groups. This enabled to show the influence of the structure of amidino group and aglicone on activity.
Results: Significant support for the above mentioned hypothesis is provided by the results of biological tests such as toxicity (LD_{50}), cardiotoxicity, and antiproliferative activity against 10 different cell lines. It is found that, most of obtained amidinoanthracyclines display lower toxicity and some of them much higher antiproliferative activity. Moreover almost all of the tested compounds exhibit the possibility to overcome the drug resistance barrier of cancer cells.
Conclusions: Obtained results indicate that the transformation of the primary amino group in anthracyclines into trisubstituted amidino group may appear as the most promising way to obtain new anticancer drugs, among which a real "*magic bullet*" can be found.

Anti-HIV activity of lectins from marine invertebrates

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Background: Role of HIV envelope carbohydrates and target T-cells glycocalix is very important for understanding of mechanism of virus invasion processes. Aims: To check anti-HIV effect of next lectins: Gal/GalNAc-specific (CGL) from mussel *Crenomytilus grayanus*, Gal-specific (CVL) from sea worm *Chaetopteris variopedatus*, GlcNAc- (DTL) and GlcNAc/GalNAc-specific (DTL-A) from colonial ascidia *Didemnum ternatanum*, mannan-binding (SVL-1) and GlcNAc-specific (SVL-2) from sea worm *Serpula vermicularis*.
Methods: The data were obtained in experiments *in vitro* on C8166 T-lymphoblastoid cell line and HIV-1_{IIIB} virus. The lectins cytotoxic concentration (CC_{50}) for C8166 cells was calculated from data of the colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. An effective concentration of lectins necessary for 50% inhibition of the HIV-1 replication (EC_{50}) was determined as level of p24 viral antigen in cell lysates by Enzyme-Linked ImmunoSorbent Assay (ELISA). The effective concentration of lectin causing 50% inhibition of syncytium formation (EC_{50}) between the C8166 cells and chronically infected cell culture H9/HIV-1_{IIIB} was calculated.
Results: All lectins investigated were nontoxic for cells practically. They inhibit virus replication and syncytium formation in C8166 cells in different dose-dependent manner. DTL is more effective as HIV invasion blocker, it has maximum antiviral index (83,333).

Lectin	EC_{50}[1], mg/L	EC_{50}[2], mg/L	CC_{50}[3], mg/L	AI[4]
CGL	45.7	27.88	263	6
CVL	0.13	1.71	>500	3846
DTL	0.006	0.002	>500	83333
DTL-A	0.59	0.36	123	208
SVL-1	89.1	36.1	>500	6
SVL-2	0.24	0.57	>500	2083

[1]EC_{50} is an effective concentration in the ELISA. [2]EC_{50} is an effective concentration in syncytium formation test. [3]CC_{50} is a cytotoxic concentration in the MTT assay. [4]Antiviral index (AI) is the CC_{50}/EC_{50}[1] ratio.
Conclusions: 1) The lectins investigated block virus replication and syncytium formation in target T-cells. 2) DTL has more effective anti-HIV activity. 3) Other CVL and SVL-2 are promise as anti-HIV drugs too.

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Cytochrome P450 and Gene-activating Agents – Cholesterol Elimination and Regression of Atherosclerosis

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Background: Principal cardiovascular disorder responsible for the global rise in mortality is atherosclerotic vascular disease. Our original studies in the 1970s linked drug-caused gene activation and the induction of cytochrome P450 (P450) with elevated plasma levels of apolipoprotein AI (apo AI) and HDL cholesterol (HDL-C), powerful indicators of a reduced risk of atherosclerotic disease.
Methods: This presentation clarifies the effects of P450-enzymes and gene-activating agents on cholesterol homeostasis, the atherosclerotic process, prevention and regression of atherosclerosis, and the manifestation of atherosclerotic disease, particularly coronary heart disease (CHD), the leading cause of death in the world.
Results: Several compounds upregulate genes acting in cholesterol elimination such as apo AI, ABC (ATP-binding cassette) transporters and P450s. P450s including CYP7A1, CYP27A1 and CYP46A1 generate hydroxycholesterols which mediate the activation of cholesterol-eliminating mechanisms (see 1-3).
The progress in studies on cholesterol regulation has greatly stimulated the search for new agents with potential to regress atherosclerosis (1-3). Many xenobiotics and natural compounds activate – via for nuclear receptors including LXR, PPAR and PXR – mechanisms which eliminate excess cholesterol. The antiatherogenic effects of many compounds including statins, fibrates and cholestyramine are mediated through the actions of P450s**.** Rosuvastatin therapy which effectively reduced LDL-C and apo B and raised HDL-C and apo AI resulted in a significant regression coronary atherosclerosis. Data from statin trials revealed that HDL-C elevation by more than 7.5 % together with effective LDL-C lowering resulted in the most profound regression of atherosclerosis. The increases in HDL-C levels were found to be an independent predictor of a beneficial outcome with statin therapy (2,3).
Conclusions: P450-enzymes are essential in the maintenance of cholesterol homeostasis. Effective gene-activating agents upregulating cholesterol-eliminating mechanisms regress atherosclerosis and reduce the occurrence of fatal and non-fatal CHD and other cardiovascular events.
1. Pharmacol Toxicol 1997;81:57; 2. Ann Med 2007;39:359; 3. Eur J Clin Pharmacol 2008;64

Safety of Etanercept in Elderly Subjects With Rheumatoid Arthritis

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Objective: To report side effects (SEs) seen in a clinical cohort of patients >65 years old with Rheumatoid Arthritis (RA) treated with the TNFα blocker Etanercept and to compare the AEs rate with patients ≤ 65 years old.
Methods: Subjects with RA that started anti TNF therapy with Etanercept from November 2005 to February 2008 and referring to our Rheumatology Unit were included in this study and prospectively followed. Safety profile points included incidence rates of all side effects (SEs), defined as: adverse events (AE), serious AE (SAE, defined as a AE that required the permanent discontinuation of the therapy, malignancies and deaths), infective AE (IAE
Results: Seventy three patients were enrolled: 26 (23 females, 3 males) aged >65 years, 47 (40 females, 7 males) aged <65 years. Mean disease onset age: 57.4±1.7 years in patients ≤65 years old, 60.4±3.2 years in patients >65 years old ($p>0.05$). Mean age at beginning of TNF blocker was 57.8±6.9 yrs in patients ≤65 years old and 68.2 ±3.2 yrs in patients >65 years ($p<0.05$). The duration of the anti TNF treatment was 19.9±16.9 and 18.6±6.3 months, respectively ($p>0.05$). In the whole population 29 SEs were observed (6AE, 17 IAE, 6 SAE), and led to temporary/permanent withdrawal in 38.4% of cases.
The rate of patients presenting SEs was 38.46% (AE 10%, IAE 50%, SAE 40%) of >65 years old versus 36.17% (AE 26.3%, IAE 63.1%, SAE 10.5%) of ≤ 65 years old patients ($p=0.408$) (table 1). The survival curves of these two groups were no significantly different (log rank test of Mantel Cox $p=0.267$)
Conclusions: In clinical experience, TNFα inhibitors are well-tolerated overall, SAEs are rare, and their risk-benefit profile strongly favours benefit. Although, long-term safety data still need to be established. Severe infections, including TB and sepsis, have been reported. Etanercept has been well-tolerated and safe overall, also in elderly patients, confirming the good safety profile of this TNF blocker

Cytotoxic Platinum(II) Complexes With Quaterpyridine Ligands As A New Class Of Topoisomerase I Inhibitors

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Background: Topoisomerase I (Topo I) has been receiving considerable interest due to its capability in regulation of cell division. The spatial arrangement of DNA before, during, and after replication is essential for cell division process. Topoisomerases regulate the linking number, twist, and writhe, ensuring that it is arranged adequately for DNA replication by breaking one or two strands of the DNA. Thus inhibition of topoisomerase by ternary (DNA–intercalator–Topo complex) formation is important in the context of drug discovery for anti-cancer treatment.
Methods: The interactions of [Pt(dpQP)]$(CF_3SO_3)_2$, [**1**, dpQP = 4′,4′′-diphenyl-2,2′:6′,2′′:6′′,2′′′-quaterpyridine] with double-stranded DNA was examined by spectroscopic, electrophoretic, and hydrodynamic methods. The spectroscopic data were analyzed with McGhee, van't Hoff, and Gibbs±Helmholtz equations. The binding mode of **1** towards the TopoI-linked DNA was further studied by molecular modeling.
Results: The binding of **1** to calf thymus DNA led to increases in the DNA melting temperature (ΔT_m = +7 °C), modest hypochromism (12% of the absorption band at λ_{max} of 358 nm). The binding constant of **1** with DNA, as determined by absorption titration, is (1.6 ± 0.2) □10^5 dm^3 mol^{-1}. A modeling study on the interaction between **1** and TopoI-linked DNA revealed that **1** intercalates to TopoI-DNA in a similar manner to topotecan (known topoisomerase I inhibitor), and exhibits a strong binding interaction. As determined by MTT assays, **1** exhibited moderate cytotoxicities toward several human cancer cell lines (KB-3-1, HepG2, and HeLa). According to confocal microscopic and flow cytometric studies, **1** induced apoptosis (70%) in cancer cells with <5% necrosis detected. Notably, **1** at concentrations > 25 □M inhibited the Topo I-mediated relaxation of DNA.
Conclusions: Cyclometalated platinum(II) complex with quaterpyridine ligand bind to DNA with binding constants ~10^5 mol^{-1} dm^3 and exhibit comparable cytotoxicities toward a series of human carcinoma cell lines with cisplatin; can induce apoptotic cell death in human carcinoma cells presumably by stabilizing the ternary, DNA–intercalator–Topo complex.

Antiviral Chemotherapy In HTLV-1 Infection: Highlights From In Vitro Studies

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Background: Human T lymphotropic virus type 1 (HTLV-1) is associated to various pathologies. The most common characterized pathologies are adult T cell leukaemia (ATL), cutaneous T cell lymphoma (CTCL)], and neurological disease; tropic spastic paraparesis (TSP/HAM)]. Therapeutic treatments for HTLV-1-related pathologies are still limited and the classical chemotherapeutic approach has failed. A serious of studies has been recently carried on to define the effects of nucleoside analogues and carbohydrate binding agents (CBAs) in HTLV-1 in vitro infection.
Methods: Quantitative analysis, of both a cell-free RT- inhibitory and cell-to cell transmission assays was performed to determine the effect of known nucleoside analogues (AZT,3TC, Tenofovir) and also a newly synthesized family of phosphonated nucleoside compounds (PCOANs). On the other side Carbohydrate-binding agents (CBAs) such as the *Hippeastrum hybrid* agglutinin (HHA), *Urtica dioica* agglutinin (UDA), and mannose-specific antibiotic Pradimicin A (PRM-A) were tested for their ability to block HTLV-1 transmission both to PBMC from a number of different normal adults or to lymphoid cell lines.
Results: PCOANs, completely inhibited cDNA elongation at concentrations close to that necessary for tenofovir to exert a similar effect. PCOANs slightly induced toxicity levels similar to that of tenofovir and lower than that of azydothymidine. CBA were able to efficiently prevent cell-to-cell HTLV-1 transmission at non-toxic concentrations as evidenced by the lack of appearance of virus-specific mRNA and of the viral protein Tax in the acceptor cells.
Conclusions: Overall, these results indicate that the family of PCOANs includes potential candidate compounds for ensuring a long lasting control of HTLV-1 infection. The anti-HTLV-1 properties of the CBAs highlight the importance of the envelope glycols in events underlying HTLV-1 passage from cell to cell and indicate that CBAs should be further investigated on their potential to prevent HTLV-1 infection, including mother-to-child virus transmission by cell-to-cell contact through breast milk feeding.

Calcium-Releasing Agent Exhibits Bioactive Effects In Endodontic Therapy

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Background: Mineral trioxide aggregate (MTA) that is mainly composed of dicalcium silicate, tricalcium silicate, tricalcium aluminate, and gypsum, has been effectively used for endodontic applications. That resulted in the favorable healing involving the hard tissue regeneration by periodontal ligament (PDL) fibroblasts that cover the surface of tooth root. However, it has been unclear how MTA contributes to such healing. Therefore, we aimed to clarify the mechanism of MTA to influence human periodontal ligament fibroblasts (HPLF) *in vitro*.
Methods: Two HPLF populations were isolated and generated from the healthy premolars of a 22-year-old female, and a 14-year-old male who visited Kyushu University Hospital for extraction, and the cells were maintained in 10%FBS/αMEM (Gibco-BRL, Grand Island, NY). All procedures were performed in compliance with the regulations of Kyushu University. White ProRoot MTA (DENTSPLY Tulsa Dental, Johnson City, TN) was mixed with sterile water according to the manufacturer's instructions, dispensed into plastic lids of 1.5ml micro-centrifuge tubes, and placed in a humidified incubator (37°C) for 12hr. MTA discs (ϕ9mm and 1mm thickness) were then rinsed with αMEM, placed in 24-well culture plates (1disk/well), and subjected to the cultures with HPLF.
Results: SEM observation showed attachment of HPLF onto MTA discs within 24hr. MTA did not inhibit the cell proliferation, and furthermore up-regulated expression of bone-related genes, *osteopontin* and *osteocalcin* in HPLF within 14days, and eventually induced mineralization in HPLF around MTA after 4wk of culture. MTA furthermore exhibited calcium release into the culture media. Then $CaCl_2$ treatment not only stimulated mRNA expression of *osteopontin* and *osteocalcin*, but induced mineralization in HPLF, while $MaCl_2$ treatment did not show any significant effects. Consequently, HPLF treated with MTA and $CaCl_2$ definitely increased *bone morphogenic protein 2* (*BMP2*) gene expression.
Conclusions: 1) MTA possesses the biocompatibility for HPLF. 2) MTA induces the osteogenic differentiation of HPLF through up-regulated BMP2 expression via the calcium release. Therefore, 3) MTA is a bioactive material in endodontic treatment.

Antimicrobial Resistance Among Re Treatment TB Patients; Sri Lankan Experience

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Background: The successful treatment of tuberculosis (TB) depends upon the selection of an effective chemotherapeutic regimen. Although the clinical accuracy of drug susceptibility test (DST) has been debated, it produces reliable results for susceptibility to isoniazid, rifampin and streptomycin. Therefore DST is essential for detecting drug resistant TB and designing effective regimens for treating individual patients. In Sri Lanka, the DST is performed only for first line anti-tuberculosis drugs. Aim; to determine the resistance patterns of *Mycobacterium tuberculosis* isolated from re treatment TB patients to first and second line anti tuberculosis drugs.
Method: The study population consisted of 131culture positive TB patients admitted for re-treatment (38 relapses, 92 defaulters, 1 treatment failure) to Chest Hospital, Welisara. Sputum samples were decontaminated using standard sodium hydroxide – sodium citrate method. DSTs were done for 12 anti tuberculosis drugs with actively growing 4-week-old cultures grown on Middle brook 7H-10-agar using the agar proportion method.
RESULTS:
Table 1; Pattern of Drug resistance among tested isolates

	Number
Susceptible to all drugs	63
Resistant to one/more first line drugs	20
Resistant to one/more second line drugs	17
Resistant to first & second line drugs	28

Table 2: Percentage resistance to anti tuberculosis drugs

Drug	No.of resistant isolates	% of resistance
Isoniazid (INH)	16	12.2
Rifampin (RMP)	10	7.6
Streptomycin (SM)	13	9.9
Ethambutol (EMB)	19	14.5
Pyrazinamide (PZA)	16	12.2
p-aminosalicylic acid (PAS)	20	15.3
Ethionamide (ETA)	15	11.4
Cycloserine (CS)	15	11.4
Kanamycin (KM)	9	6.8
Ciprofloxacin (CIP)	7	5.3
Viomycin (VM)	2	1.5
Rifabutin (RIB)	1	0.7

Table 3: Antimicrobiol resistance pattern of multi-drug resistant MDR strains

Anti-TB drugs	Number of resistant strains
INH+RMP+PZA	1
INH+RMP+CS	1
INH+RMP+PZA+KM+CS	1
INH+RMP+PZA+EMB+SM+KM+CS+PAS	1

CONCLUSIONS: 1) High rate of acquired drug resistance and the wide variation in drug sensitivity patterns was observed.2) Results in our study indicates the necessity to have an antibiotic sensitivity test before instituting treatment for recurrent TB patients.

Pharmacokinetic-Pharmacodynamic Modeling Of Aliskiren Effects On Biomarkers Of The Renin-Angiotensin System In Humans

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Background: Drugs targeting the renin-angiotensin system (RAS) are an important therapeutic class for the management of several cardiovascular disorders. Aliskiren is the first approved orally active renin inhibitor and exhibits a dose-dependent antihypertensive effect. The purpose of this study is to develop a semi-mechanistic pharmacokinetic-pharmacodynamic (PK-PD) to evaluate aliskiren effects on several biomarkers of RAS in humans.
Methods: Mean plasma renin activity (PRA) and plasma concentrations of aliskiren, active renin (AR), angiotensin-I (ANG-I), and angiotensin-II (ANG-II) were extracted from a published 3-way crossover, placebo-controlled study. Healthy male volunteers (n=18) received either placebo or 20mg enalapril followed by 2 oral doses of aliskiren (40 and 80mg, or 160 and 640mg) once daily (6-day washout period). The final PK-PD model was fitted to the data in 3-stages: 1) multiple-dosing PK were modeled and fixed during subsequent stages, 2) single-dose PD were characterized, and 3) single- and multiple-dose PD were fitted jointly. All model parameters were estimated using the maximum likelihood method in S-ADAPT (v.1.51).
Results: A two-compartment model with nonlinear elimination and distribution best described aliskiren disposition. AR increased in a dose-dependent manner following the administration of aliskiren, which was described by an indirect stimulatory response model in conjunction with an empirical sub-model of functional adaptation. In contrast to AR, PRA decreased in a dose-dependent manner and was maximally inhibited within 1 hour after aliskiren administration. This response was well captured with a direct inhibitory E_{max} model, and the estimated aliskiren concentration producing 50% inhibition of PRA was 0.66ng/mL, which is similar to *in vitro* estimates (0.33ng/mL) after correcting for plasma protein binding. Inhibition of ANG-I and ANG-II paralleled the changes in PRA, and ANG-I and ANG-II remained linearly correlated throughout the study. A reduced model was also developed excluding ANG profiles, which successfully described AR and PRA profiles after multiple-dosing.
Conclusions: An integrated PK-PD model of aliskiren was developed which is consistent with the pharmacology of renin inhibition. The final and reduced models test current hypotheses of RAS inhibition by direct renin antagonism and may prove useful in the future clinical development of renin inhibitors.

Four Decades In Selegiline Research; Historical Aspects, Further Perspectives

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The most outstanding discovery of the Hungarian drug research still is selegiline (/-/-deprenyl), a selective irreversible inhibitor of monoamine oxidase-B (MAO-B). Our aim was to develop a potent antidepressive agent. However, because of the “cheese reaction”, appeared with other MAO inhibitors, similarly to those, it has fallen into disrepute. Selegiline survived the shock of “cheese reaction”, due to its dopamine potentiating and antioxidant capacity. It became the gold standard of MAO inhibitors possessing a wide range of pharmacological activities, many of them are not related to its MAO-B inhibitory potency. It protects the effects of neurotoxins and it combats oxidative challenge, factors leading to neurodegenerative diseases. In high concentration it has pro-apoptotic activity, while in very low concentration range (10^{-9} to 10^{-13} M) inhibits apoptosis and interferes with both the intrinsic (mitochondrial) and extrinsic (death receptor) apoptotic pathways. Metabolic conversion of selegiline results partly in propargylamine containing metabolites, most probably responsible in its antiapoptotic property, including the transcriptional and translational changes. Pharmacokinetic studies revealed that selegiline undergoes intensive first pass metabolism, so its pharmacological effects are strongly influenced by the routes of its administration. Presently, in a formula of transdermal patch, selegiline in a usual oral dose, administered to parkinsonian patients, started to be used as an antidepressive agent. Research is carried out presently to identify the metabolite, responsible for neuroprotection. Because of the concentration-dependent neuroprotective effects of selegiline an optimal dosing schedule is needed to diminish undesired adverse events, with unaltered or improved neuroprotective activity

Tetracyclines: A Historical Pitfall And Additional Concept On The Treatment Of Rickettsial Diseases

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Background: A febrile disease thought to be Scrub typhus(Tsutsugamushi disease) appeared in China in 313 A.D. The first scientific report was made by Bealtz and Kawakami in 1879. This old and historical disease was highlighted at the 13th ICID (June 2008, ML), in a symposium entitled “A neglected pathogen *Orientia tsutsugamushi”.* In it, the author defined the following problems. 1) This disease must be recognized as a re-emerging infectious disease. 2) Chemotherapy - Empiric treatment of rickettsioses is initiated with Doxycycline (DOXY) as the world standard, however surprisingly except for Japan. This fact suggests a great historical pitfall. In this report we present the current epidemiology of scrub typhus briefly and focus on the treatment of scrub typhus and Japanese spotted fever (JSF).
Method: Epidemiologic data was collected in the MEDLINE database and the NIH Website of each country. But most of the data was collected through personal communication. The clinical data was based on our original investigations.
Results: Epidemiology: Scrub typhus is known to be distributed throughout the tsutsugamushi triangle. The re-emerging increase of the cases was reported in Korea 6663 (2005), in Thailand 5094 (2001) and in Japan 957 (1984). More than 20 cases of travel acquired patients were reported worldwide. **Chemotherapy**: DOXY is the main antibiotic in almost all countries (Korea, Russia, China, Taiwan, Thailand, India etc.). Minocycline (MINO) is not used except for Japan. However drug resistance to DOXY has been reported. On the other hand, MINO is the standard treatment for Tsutsugamushi disease in Japan. More than 4000 cases were treated with MINO in the last ten years, and drug resistance was not reported. With a single dose of 200 mg of MINO, 90% of the patients were afebrile within 24 hrs. To clarify the mechanism, we investigated the cytokine modulation induced by tetracyclines in vivo and in vitro studies (unpub. data). Quinolones are effective for spotted fever rickettsiae though tetracyclines are more sensitive. So, the author recommended the combination treatment with MINO and Quinolone for the severe cases of JSF. We think this method is applicable for treatment of other rickettsiosis.
Conclusion: 1) We proved the usefulness of MINO which is standard only in Japan among the world. 2) This fact supports the paradigm change of double bullets (DOXY and MINO), as the global standard for the treatment of Rickettsial diseases. 3) Clinicians must pay more attention to scrub typhus and other rickettsioses, and consider the double bullets to prevent travel acquired rickettsioses.

Infectious Pregnancy Complications

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Epidemiological reports estimate that 7.7 million perinatal deaths occur annually worldwide, including 4.3 million that take place in late pregnancy, while the remaining neonates die in the first weeks of life. Reports attribute the majority of these consequences to infections of the fetus in utero. Infections during pregnancy affect the mother and often some infections may be transmitted to the fetus in utero, during the intrapartum period or, postnatally, with potentially serious consequences. Many infections have been linked with increased risks of premature delivery and low birth weight, and associated morbidity and mortality of both mother and child. Acute or chronic specific infectious diseases may be contracted during the course of pregnancy, and conception may occur in women already subject to an infection. The coexistence of pregnancy may aggravate the risk to maternal life in cases of the more serious of these diseases. In pregnancy most infections are no more common, nor more serious than in a non-pregnant population of women of similar age. The effects on pregnancy depend on the degree of pyrexia, its duration, and the stage of fetal development when it occurs. Mild exposures during the preimplantation period, and more severe exposures during embryonic and fetal development often result in miscarriage, premature labor, growth restriction, or stillbirth. Hyperthermia may also cause a wide range of fetal structural and functional defects, with the central nervous system (CNS) being most at risk. While there is a greater incidence of neonatal morbidity and mortality with transmitted infections, not all maternal infections lead to transmission to the fetus, nor does transmission to the fetus lead to disease or sequelae. During the puerperium, parturient women are particularly susceptible to serious infections of the genital tract and childbed fever remains one of the most important causes of maternal death. Infections in pregnancy may be viral, bacterial or protozoal, affecting both mother and fetus. Some of the infections cause fevers, while others may not; this chapter will concentrate on infections resulting in maternal pyrexia, and some other infections which may not result in maternal pyrexia, but have important implications for the pregnancy and the fetus.

Let Food Be Thy Medicine And Medicine Be Thy Food

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Background: The relationships between food and health have been recognized centuries ago. Hippocrates quote "let food be thy medicine and medicine be thy food" dates more than 2500 years back. Early nutrition research resulted in cures for many deficiency-based diseases. Recent scientific advances have blurred the line between food and medicine, as scientists identify bioactive food components that can reduce the risk of chronic disease, improve quality of life and promote general health.
Methods: Literature survey and updates on the issue of functional foods have been conducted.
Results: Positive health benefits from food components not considered nutrients in the traditional definition have been identified. This has resulted in the introduction of the term *"functional foods*" (nutraceuticals). Functional food is any food claimed to have a health-promoting and/or disease-preventing property beyond the function of supplying nutrients. Some of the potential benefits of functional foods include reducing risk of hypertension, reduced risks of cardiovascular diseases, and benefits of antioxidants in scavenging free radicals. Functional foods can take many forms (e.g. conventional foods with bioactive components, fortified or enhanced foods specifically created to reduce disease risk). Several promising bioactive compounds in foods of pant origin including legumes, cruciferous vegetables, flaxseed, barley, soy, berries, apples, coffee, tea, and oat, have been identified and studied. Examples of functional food components currently marketed, select functional foods, key components, and potential health benefits are discussed. Some challenges facing functional foods include the need for scientific, regulatory, and business frameworks, objective evaluation of data for efficacy and safety, communication of the findings to consumers, and providing incentives to encourage R & D of these novel food products.
Conclusions: Developing functional foods to improve public health requires contributions from on-going research and modifications to the current regulatory framework to facilitate the review of new functional components and their health claims. It is also imperative to communicate the correct information to the consumers.

Erythropoietin: Bold Directions And Strategies For A "Magic Bullet" Destined For Neurovascular Disease

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Implications for the clinical utility of erythropoietin (EPO) in the nervous and cardiovascular systems continue to reach far beyond the sole treatment of systemic anemia, but the development of EPO as a safe and non-toxic therapy that does not breach borders into neoplastic growth rests heavily upon the elucidation of the cellular pathways governed by this agent. Here we discuss novel strategies for the development of EPO as a cytoprotectant *in vitro* and *in vivo* during oxidative stress. Apoptotic neuronal and vascular protection by EPO occurs within a specific pre- and post-treatment therapeutic window that requires the activation of protein kinase B (Akt1), since loss of Akt1 activity through direct pharmacological blockade of Akt1 or through the gene silencing of Akt1 expression prevents cytoprotection by EPO. Yet, intimately coupled to the robust protection by EPO are a series of unique cellular mechanisms that control STAT, ERK 1/2 proteins, *wingless* (Wnt), and the intracellular trafficking of the Forkhead transcription factors such as FoxO3a. For example, in a series of sequential steps, EPO maintains inhibitory phosphorylation of FoxO3a and blocks FoxO3a proteolysis to foster cell survival. Second, EPO promotes the binding of FoxO3a to 14-3-3 protein through Akt1 to sequester FoxO3a in the cytoplasm to prevent transcription of apoptotic proteins. Third, gene silencing of FoxO3a during oxidative stress significantly increases cell survival, but does not synergistically improve the cytoprotective capacity of EPO, illustrating that EPO relies upon the specific blockade of the FoxO3a pathway. Fruitful development of EPO as a "magic bullet" destined for a host of neuronal and cardiovascular disorders that avoids toxicity and maximizes therapeutic efficacy will continue to depend heavily upon the elucidation and targeting of the cellular mechanisms governed by EPO in the nervous and vascular systems.

The Critical Functions Of Biliverdin Reductase (BVR) In Insulin/Insulin Growth Factor-1 (IGF-1) And MAPK Signaling Pathways: A Potential Therapeutic Application In Treatment Of Diabetes And Cancer

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Background: Among eukaryotic proteins, the human(h) BVR has a more diverse and expansive spectrum of functions in the cell than any other protein. The breadth of its functions was discovered in recent years and lends itself to the development of peptide-based technology to combat diseases that are associated with disruption of normal kinase-mediated functions such as diabetes and cancer.
Methods: In vitro and in cell experiments, using human embryonic kidney cells were employed to examine the role of hBVR in insulin receptor kinase/IGF-1/MAPK-regulated signaling and regulation of gene expression.
Results: Data gathered identified the ability of hBVR, its variants and small fragments, in modulating cell signaling and, hence, the wide range of functions that are regulated by protein kinases. These functions include growth, differentiation, gene transcription and metabolism. Regulation of glucose uptake, induction of heme oxygenase-1 (HO-1), and cytokine and Toll-like receptor signaling were identified as potential target candidates for hBVR-based therapeutic strategies. Because hBVR blocked free radical-promoted apoptosis, regulation of its activity presents a novel drug development strategy to prolong cell survival. hBVR is nearly as effective as IGF in activating the MEK-1-ERK axis. Two eight-residue peptides, one flanking P^{165} and another containing S^{230} block or activate, respectively, ERK, by IGF and PKC-ζ, by TNF-α. This suggests their utility as effectors of cell cycle progression and cell differentiation. Furthermore, the peptide: KYCCSRK could specifically bind hematin, which offers a rational approach to design compounds, based on the ligand-binding property, for delivering heme or synthetic heme analogues to induce or inhibit heme-regulated gene expression, including HO-1.
Conclusions: Because hBVR-based seven or eight-residue peptides can effectively modulate cell-signaling networks, they bear the promise of developing into powerful tools for inhibiting or potentiating transmission of extracellular stimuli that control gene expression and cellular functions. As additional functions of hBVR in cell signaling are uncovered, the prospect of the utility of hBVR-derived structures in therapeutic settings becomes increasingly more realistic.
Disclosure Statement: This work was supported by NIH grants R01ES-04066ES and R01ES-12187. I would like to thank Philip Gibbs for help in preparation of this abstract. Based on reports in Maines MD, Phys, 2005; Lerner-Marmarosh N, et al, PNAS, 2005; Miralem T, et al, JBC, 2005; Gibbs PE and Maines MD, Int J Cancer, 2007; Lerner-Marmarosh N et al, Faseb J, 2007; Maines MD, et al, JBC, 2007; and Lerner-Marmarosh N, et al, PNAS, 2008.

Intrapericardial Cisplatin Treatment Prevents Effectively The Recurrence Of Neoplastic Pericardial Effusion

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Background: The differential diagnosis of neoplastic (NE) vs. radiation induced pericardial effusion (RE) in malignancies is the prerequisite of an adequate intrapericardial and/or systemic treatment.
Methods: Out of 260 pts undergoing pericardiocentesis, 42 pts with pericardial effusion (69% m, mean age 58,8±13,2 y) were identified as NE,15 pts (66% fem, mean age 54,7±12.4 y) as RE. The aetiological assessment was highly effective because it was assisted by pericardioscopy, epi- and pericardial biopsy and pericardial cytology. Pericardial effusion and biopsy analyses included biochemistry, cytology, (immuno)histology, and PCR.
Results: In NE we identified: lung cancer, 52,4%; breast cancer, 19,0%; Hodgkin's disease, 4,8%; oesophageal cancer, 2,4%; mesothelioma, 2,4%; colon cancer, 4,8%; and undifferentiated cancer of unknown origin, 14,2%. In RE 11 pts had previous breast cancer, 4 pts bronchus carcinoma but the PE was negative for them.
NE were treated with intrapericardial cisplatin (single instillation of 30 mg / m² for 24 h) in addition to the tumour-specific systemic chemotherapy. It prevented recurrence of pericardial effusion during the first 3 months of the follow-up in 92,8%, and after 6 months in 83,3% of the pts. Lung cancer patients had fewer effusion relapses at the 6 months follow-up (4,5%) than breast cancer patients (37,5%)($P<0,05$). Myocardial ischemia occurred after 1/42 cisplatin instillations, but there were no other complications.
RE received 500mg / m² triamcinolonacetate (Volon A) intrapericardially followed by 6 months oral treatment with colchicin (3x0,5 mg). Recurrence of effusion was prevented in 13 of 15 cases (86,6%) after 3 and 6 months. With the Touhy needle and the Marburg Attacher we reach now small effusions for intrapericardial diagnosis and treatment.
Conclusions: 1) Intrapericardial treatment with cisplatin prevents recurrences of NE effectively. The treatment was more successful in lung than in breast cancer pts. 2) In RE sclerosing treatment with triamcinolonacete was equally effective. 3) Pericardioscopy adds considerably to adequate diagnosis and consecutive treatment.

Design Of Folate-Linked Liposomal Doxorubicin To Its Antitumor Effect In Mice

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Background: Tumor cell targeting is a promising strategy for enhancing the therapeutic potential of chemotherapy agents. Polyethylene glycol (PEG)-coated (sterically stabilized) liposomes show enhanced accumulation on the surface of tumors, but steric hindrance by PEGylation reduces the association of the liposome-bound ligand with its receptor. To increase folate receptor (FR)-targeting, we optimized the concentration and PEG spacer length of folate-PEG-lipid in liposomes.
Methods: Three types of folate-linked liposomal doxorubicin (DXR) were designed and prepared by optimizing the concentration and PEG-spacer length of folate-PEG-lipid in PEGylated or non-PEGylated liposomes, and by masking folate-linked liposomes where the folate ligand is "masked" by adjacent PEG spacers. The liposome targeting efficacy was evaluated *in vitro* and *in vivo*.
Results: The effects of PEG-spacer length and ligand density on folate receptor-targeted liposomes were evaluated. In human oral carcinoma KB cells, which overexpress FR, modification with sufficiently long PEG spacer and a high concentration of folate ligand to non-PEGylated liposomes increased the FR-mediated association and cytotoxicity more than with PEGylated and masked folate-linked liposomes. On the contrary, in mice bearing murine lung carcinoma M109, modification with the folate ligand in PEGylated and masked folate-linked liposomes showed significantly higher antitumor effect than with non-PEGylated liposomes irrespective of the length of time in the circulation after i.v. injection.
Conclusions: 1) Folate ligands with 0.25 mol% and sufficiently long PEG spacers ($F\text{-}PEG_{5000}$-DSPE) of the folate ligand of liposomes without PEG-coating increased the folate receptor association and cytotoxicity compared with those with PEG-coating and masking folate-linked liposomes *in vitro*. 2) On the contrary, folate-linked and masking folate-linked liposomes showed a higher tumor killing effect than folate-linked liposomes without PEG-coating *in vivo*. The results of this study will be beneficial for the design and preparation of ligand-targeting carriers for cancer treatment.

Competitive Interactions In *Escherichia Coli* Populations: The Role Of Bacteriocins

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Introduction: Bacteriocins are highly toxic, naturally occurring antimicrobials found in all lineages of bacteria. Hundreds of these toxins have been identified each active only against its close relatives, thus they were proposed for drug development enabling the creation of pathogen-specific designer drugs. Bacteriocins producers were shown to competitively exclude non-producing strains, yet, the dynamics between bacteriocin producers are largely unknown. Here we studied the competitive interactions between them employing *in vitro* and *in vivo* models.
Methods: Two Escherichia coli strains were generated, each carrying a unique bacteriocin (E2 & E7). The bacteriocins encoding gene promoters were amplified, fused to reporter vectors and transferred to bacterial hosts. The reporter strains were used to monitor bacteriocin mediated induction. The bacteriocin producers were first competed against each other in a static culture environment and then employed in a murine model. Each strain was established in 7 mice and 3 mixed (E2/E7) and 4 control cages (E2/E2 & E7/E7) were generated. Cell density and killing phenotypes were monitored by fecal sampling.
Results: Using reporter gene assays we found that each bacteriocin is not only lethal to its opponent but at lower doses can also induce its expression. In the static culture the ratios of the distribution of the bacteriocinogenic populations did not change over time. In the mixed cages, no strain replacement occurred between mice harboring the experimental cages.Bacterial density fluctuated over time in the control cages while, the mixed cages showed reduced yet stable cell concentration over the sampling period.
Conclusions: We have demonstrated that due to mutual induction each population excluded its competitor by inhibiting invasion. Both models imply that bacteriocin-mediated bacteriocin induction enable producers to successfully compete and defend their niche against challenging invaders. We thus suggest that bacteriocin producing strains can potentially be used as bio-protective agents controlling the invasion of un-desired bacterial species.

The Thyroid Gland Function Assessment In Women After Mastectomy And Chemotherapy During Breast Cancer Therapy.

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Background: For many years much attention has been focused on an interaction between the breast disease and the thyroid gland function in the literature. In those studies the question whether disease changes in the thyroid gland can induces the breast disease was addressed. On the other hand there are a few works concerning the inverted question whether the breast cancer therapy, in particular after mastectomy and chemotherapy, can disturb the thyroid gland function. So, the aim of the study is to investigate the influence of the mastectomy and chemotherapy on the thyroid gland function in women after breast cancer therapy.
Materials and methods: 173 patients aged 30-80 (average 56) were included in this study. The studied group comprised 97 women after breast cancer therapy (average age 60).The control group consisted of 76 patients (average age 55). 75 patients after mastectomy of the studied group were additionally treated with chemotherapy (CMF: cyclophosphamide, methotrexate, 5-fluorouracil;FAC: 5-fluorouracil, adriamecine, cyclophosphamide). The following methods were used to carry out the research: the USG method was applied to evaluate thyroid morphological condition in women after mastectomy and chemotherapy; the Color-Doppler technique was used for dynamic presentation and fine- needle aspiration biopsy: examination of the thyroid functional state by measuring the TSH, fT_3, fT_4 hormone concentration and the level of antithyroid antibodies.
Results: the average concentration of antithyroid antibodies: anty-TPO and anty-Tg was found significantly higher in the studied group of women after chemotherapy, comparing with the control group. The level of fT_3 hormone concentration was comparable in all investigated groups. Nevertheless, the average concentration of TSH was found higher in women after mastectomy and chemotherapy and as a consequence leading to hypothyroidism.
Conclusion: Taking into consideration the high level of the concentration of antithyroid antibodies: (anty-TPO and anty-Tg), which lead to destruction of the thyroid gland tissue, the thyroid gland function of the women after mastectomy and chemotherapy should be monitored morphologically as well as functionally.

Pulmonary Drug Delivery System For Efficient Treatment Of Tuberculosis

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Background: Inhalable aerosol particles containing tubaculostatic agents are useful for the treatment of initial infection with Mycobacterium tuberculosis in the lung so that multiple drug resistant strains do not occur. Mycobacteria, which are resistant to biological digestion in macrophages, continuously grow in the phagosomes of alveolar macrophages using macrophages as incubators. Hence, the biocompatible and biodegradable microspheres containing anti-tuberculosis agents will be efficient for therapy of tuberculosis, if they are taken up well by alveolar macrophages through phagocytosis.
Methods: We have prepared PLGA microspheres containing the anti-tuberculosis agent rifampicin (RFP), by spray drying method, and they were administered to NR8383 cells derived from rat alveolar macrophages. RFP-loaded PLGA microspheres were administered by inhalation to rats which were infected with mycobacterium tuberculosis every day for 2 weeks and their killing effects against tubercle bacilli were evaluated.
Results: (1) NR8383 cells phagocytosed most efficiently the PLGA microspheres with a diameter of about 3 μm, and phagocytosis attained the maximal level after 4 hrs, (2) the phagocytosis of PLGA microspheres stimulated phagocytic activity of macrophages, (3) the amount of RFP taken up by macrophages in a form of particles was about 20 times greater than that administered in a form of solution, and (4) RFP in macrophages efficiently killed BCG, used as a model of *Mycobacterium tuberculosis*, taken up by phagocytosis. Also we have shown that the RFP-loaded PLGA microspheres can reach deep in lung by inhalation and have shown the therapeutic effects *in vivo*.
Conclusions: It is possible that tuberculosis can be overcome efficiently by administration of anti-tuberculosis agents in a form of inhalable PLGA microspheres to alveolar macrophages.

A New Application Of Granulocyte Colony-Stimulating Factor (G-CSF) In The Female Reproductive Medicine - For Prevention Of Luteinized Unruptured Follicle (LUF) -

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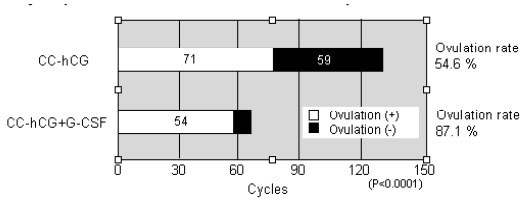
Background: G-CSF is detected in follicles and it shows a peak concentration in serum on a few days before ovulation. The expression level of G-CSF mRNA in the follicular wall at similar phase is 10-fold greater than other phases. These findings indicate that G-CSF plays an important role in the mechanism of follicle rupture, ovulation. LUF is an ovulation disorder that shows follicular growth and luteinization but lacks follicle rupture. We have conducted a clinical trial to induce ovulation by G-CSF for the patients of LUF.
Methods: Patients who received Clomiphene (CC) - human Chorionic Gonadotropin (hCG) treatment for ovulation induction and showed LUF at the last induction cycle participated in this study with informed consent. In addition to CC - hCG, G-CSF 100 μg was administered at 24 - 48 hours before hCG administration in 62 cycles of 56 patients, considering the natural cyclic changes of serum G-CSF. Ovulation was then confirmed by ultrasonography.
Results: The total numbers of LUF cycles before G-CSF treatment were 59 of 130 cycles (45.4%). Ovulation was successfully induced in 54 cycles (87.1%) with G-CSF, which is significantly higher than the cycles without G-CSF (P<0.0001). Pregnancy was confirmed in 4 cases of G-CSF treated cycles.



Conclusions: G-CSF administration during CC - hCG treatment is very effective to prevent LUF. Since there are no other treatments at present, G-CSF must be used for LUF patients as the first choice.

Argyrin A A New Type Of Proteasome Inhibitor With Potent Anti-Proliferative And Anti-Angiogenic Activities

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The cyclin kinase inhibitor $p27^{kip1}$ acts as an important tumor suppressor protein in a variety of human cancers. Its expression levels closely correlate with the prognosis of the affected patient and also predict the outcome of various treatment modalities. While many tumors express low levels of p27 its genomic locus is rarely mutated in cancer cells. We previously showed that ubiquitin dependent turnover of p27 is part of the progression sequence of intestinal cancers. Using a mouse system in which p27 turnover is partly inhibited we were able to demonstrate that interference with p27 degradation results in a block to tumor cell de-differentiation in the intestine. Based on these results we decided to conduct a small molecule screen to identify substances which block p27 degradation. We identified Argyrin A, a natural compound derived from myxobacteria as a potent inducer of p27 expression in a variety of cancer cells. Treatment with Argyrin A induces apoptosis in human colon carcinoma cells *in vitro* and in xenotransplant tumors derived from these cell lines *in vivo*. Moreover Argyrin A also leads to a dramatic reduction of tumor vasculature in xenotransplants and an inhibition of HUVEC tube formation *in vitro*. All of these effects are dependent on p27 expression as cells derived from p27 knockout mice or cells in which p27 expression was lowered by siRNA treatment did not respond to Argyrin A. Interestingly cells in which the activity of the proteasome was lowered using siRNA specific for the beta1,2,5 subunits also displayed cellular phenotypes only when p27 was expressed in these cells.
Our results point towards a central role of p27 in controlling the cellular responses connected with proteasome inhibition. They also point towards the existence of signalling events downstream of the proteasome which use distinct proteins to mediate specific cellular phenotypes. The high efficiency with which Argyrin A targets cancer cells and tumor vasculature combined with its very low toxicity *in vivo* makes a p27 directed therapy using Argyrin A worthy of further clinical development.

The Natural Estrogens And Mycoestrogens: From Simple Cell Proliferation To Transcriptional Effects On Cytochrome P450 1A1 And 1B1 Expression In Human Breast Cancer Cells

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Background: In last decades much focused on compounds which acting as endocrine disrupters and among others mycoestrogens attracted much scientific attention.
Aims: 1- to study the mycoestrogen Zearalenone's (ZEA) toxicokinetics (TK) in animals and human in order to uncover potential target receptors other than estrogen receptors; 2- to evaluate any effect of estrogens and mycoestrogens at transcriptional level, to find possible approach for therapeutic purposes.
Methods: To study the biotransformation of ZEA, incubation of ZEA with hepatic sub-cellular fractions of animals and human was conducted. Real-time PCR analysis was also performed to determine the enzyme(s) which are involved in biotransformation of ZEA. Finally to determine the effect of the E_2 and mycoestrogens on Cytochrome P450 1A1 and 1B1, qPCR analyses were performed.
Results: the biotransformation studies revealed that ZEA is converted predominantly into α-Zearalenol as potent estrogenic metabolite in pig and human and into β-Zearalenol as the weakest estrogenic metabolite in chicken and rat. We demonstrated that 3α-HSD and 3β-HSD are expressed in tissues that are exposed to ZEA and consequently ZEA metabolites are formed.
Exposing MCF-7 cells against E_2 and ZEA reduced EROD activities significantly. Subsequent qPCR studies showed that the treatment of MCF-7 cells with E_2 and mycoestrogens resulted to down-regulation of CYP 1A1 and up-regulation of CYP 1B1 in mRNA level.
Conclusions: 1) as α-ZOL is the major metabolite of ZEA in human and pig, thus the reason of being sensitive to ZEA could be justified and equally as enzymes which are involved in this processes were identified, therefore any inhibitor of mentioned enzymes could be potential therapeutic agent in ZEA intoxication; 2) as CYP 1A1 and 1B1 are oxidative enzymes in steroids metabolism and any alteration in expression of these two could disturb the balance of the catechol estrogens formation, therefore prevention of any potential effect of ZEA and its metabolites on transcriptional level could be count as a novel approach in prevention of carcinogenesis.

Potential Of JAK/STAT Inhibitors For Treating RA

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Background: IL-1, IL-6, IL-2, IL-7, IL-12, IL-15, TNF-α, and the interferons are proinflammatory cytokines that contribute to cartilage destruction in RA, in part, by maintaining synoviocyte survival. These cytokines may also induce chondrocyte apoptosis. Whereas IL-1 and TNF-α bind to IL-1R and TNFR1/TNFR2, respectively, where they activate the SAP/MAPK pathway, IL-6, IL-2, IL-7, IL-12, IL-15 and interferons are known to bind to Type I and Type II cytokine receptors and activate the JAK/STAT pathway. In this study, cultured human chondrocytes were used to determine which SAP/MAPKs and/or STAT proteins were specifically activated by human recombinant TNF-α.
Methods: Chondrocytes were liberated from human osteoarthritic (OA) cartilage by enzymatic digestion. After growth in monolayer culture, chondrocytes were grown in high-density microcultures which were previously shown to maintain the chondrogenic phenotype (Malemud CJ *et al. Cells Tissues Organs* 2003; 174: 34-48) and treated with TNF-α (10 ng/ml) for 24 hr after which cell lysates were collected at 1, 5, 30 and 60 min. SDS/PAGE combined with Western blotting determined the extent to which TNF-α altered STAT, p38 kinase (p38), JNK1(p46), JNK2 (p54) phosphorylation and protein.
Results: Within 1 min TNF-α caused specific phosphorylation of STAT3. STAT3 was maintained in the activated state for up to 30 min after which phosphorylated STAT3 was no longer detectable. TNF-α also gradually activated p38 where maximal phosphorylated p38 was detected at 30 min. TNF-α preferentially activated JNK2 within 5 min which was maintained for up to 60 min, whereas JNK1 phosphorylation increased for up to 5 min but was no longer detectable after that time. STAT3, p38 or JNK protein levels were not altered.
Conclusions: These studies showed that TNF-α specifically activated chondrocyte STAT3 as well as p38 and JNK1, 2. Since it was previously shown that STAT3 was critical in order for RA or OA synoviocytes to survive (Krause A *et al. J Immunol* 2002; 169: 6610-6) the results of these *in vitro* studies suggested that a JAK2 inhibitor such as AG-490 may be useful for determining if STAT3 inhibition alone will result in NF-κB activation and induce chondrocyte apoptosis or whether additional specific p38 and JNK small molecule inhibitors can counteract TNF-α induced chondrocyte STAT3 activation which may be critical for limiting chondrocyte apoptosis in RA.

Spectrum And Risk For HIV-1 Associated Mutations And The Efficacy Of Antiretroviral Therapy Among Infected Pediatric Patients

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Background: Introduction of combined therapy with reverse transcriptase and protease inhibitors has resulted in a considerable decrease in HIV related mortality, but it has also induced the development of multiple drug resistant HIV-1 variants. The few studies on HIV mutagenesis in infected children have not evaluated the impact of related mutations on pediatric HIV disease.
Methods: 42 HIV-1 infected children were enrolled and followed in the Robert Wood Johnson Pediatric Infectious Disease Clinic in New Brunswick, NJ, USA since 1999 to 2007. 41(97.6%) patients were assessed and demographic and treatment-related information was recorded as well as plasma viral load, CD4 T-lymphocyte counts and HIV genotype analysis. Between 2 and 5 measurements were obtained with 6-12 month intervals and a total of 119 measurements were assessed and analyzed for 40 patients (95%).
Results: 25 male and 16 female patients were enrolled. All participants were symptomatic and had preceding treatment history with combined ARV regimens including protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI), and non-nucleoside reverse transcriptase inhibitors (NNRTI). Treatment regimens that included PIs were observed in 29/ 40 (72.5%) patients. Combined regimens did not significantly impact the incidence of NRTI and NNRTI associated mutations. Primary mutations in the protease gene increased the likelihood of plasma viral load ($\geq$10,000 copies/mL) irrespective of the child's age, duration of therapy, or presence of NRTI and NNRTI mutations, P<0.008.
13 out of 40 (44.8%) were diagnosed with PI associated major mutations at study entry. Starting PI medication (n=4) among PI naïve patients (n=11) was associated with development of PI major mutations in 25% of cases. Anaysis of 119 HIV genotype measurements showed significant association between PI mutations and HIV viral load $\geq$ 10,000 copies/mL (OR=2.84, 95% CI 1.21, 6.69).
Conclusions: Since primary PI mutations significantly increase the likelihood for high viral replication in HIV-infected pediatric patients, careful monitoring of PI resistant major mutations is important for maintaining low viral loads.

Bilirubin: An Endogenous Molecule With Antiviral Activity *In Vitro*

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Background: In 1992, Nakagami et al. hypothesized an antiviral role for biliverdin, one of the by-products of the heme-degrading enzyme heme oxygenase, against the human herpes virus-6. However, biliverdin is rapidly reduced to bilirubin (BR), a molecule endowed with strong antioxidant and antinitrosative features. Aim of this work was to study if BR may reduce the replication of herpes simplex-1 (HSV-1), cytomegalovirus (CMV) and enterovirus (EV) *in vitro*.
Methods: Monolayers of Hep-2, Vero and MRC5 cells have been infected with clinical isolates of HSV-1, CMV and EV. To mimic the different pathophysiological situations occurring during viral infection, BR (1-10 μM) alone or in the presence of saturating human serum albumin (HSA, 10 μM) was given 2 hours (h) before, concomitantly and 2 h after viral infection. In selected experiments, BR and BR-HSA were pre-incubated with HSV-1 and EV and then given Hep-2 and Vero. After 24 and 48 h of incubation, infected cells were visualized by immunofluorescence and counted. CMV DNA copies were calculated by Real-Time PCR.
Results: When given before or together with HSV-1 and EV, BR (1-10 μM) and BR-HSA, significantly reduced viral replication after 24 and 48 h of incubation. A similar effect occurred when BR and BR-HSA, as above, were pre-incubated with HSV-1 and EV and then administered to cells. Finally, when Hep-2 and Vero cells were treated with BR (1-10 μM) following viral infection, the cytoprotective effect of the bile pigment against HSV-1 infection was evident only within the first 24 h of infection, whereas the effect on EV both at 24 and 48 h. When BR (5 μM) was given MRC5 fibroblasts before and together with CMV the viral load exhibited a significant decrease only after 5 and 7 days of incubation. In search for a mechanism to explain these cytoprotective effects of BR, the hypothesis of an increased cell stress response stress has been explored. Both Hep-2 and Vero cells incubated with BR (1-10 μM) exhibited a marked activation of the proto-oncogene Akt and the kinase JNK, two pathways involved in cell survival. Ultimately, BR (1-10 μM) dose-dependently increased nitric oxide production in Vero cells.
Conclusions: Bilirubin and BR-HSA, inhibited viral replication probably by increasing cellular stress response or cytotoxic endogenous molecules such as NO.

Isoniazid: It Was Or It Is A Magic Bullet?

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In 1952, Fox (US 2596069) reported the realization of the hydrazide of isonicotinic acid (isonicotinyl hydrazide or isoniazid, INH). The drug was an intermediate in the synthesis of the thiosemicarbazone of isonicotinoylaldehyde, a compound created by Fox himself during his search for novel thiosemicarbazone derivatives with antimycobacterial properties. These compounds had been studied by Domagk in the 1940s, and one, 4-acetylaminobenzaldehyde thiosemicarbazone (TB-1/698, aminothiozone, thiacetazone), had already been marketed as an antitubercular drug under the name of Conteben.
Previous studies on the antitubercular properties of pyridine bodies – in particular, nicotinamide – also contributed to the development of INH. Chorine (1945) had found the latter compound to possess in vivo activity against infections caused by mycobacteria in animals and, at very high doses an antitubercolar activity was found in humans as well (1951-52). Substitutions of its heterocyclic group rendered nicotinamide inactive as antimycobacterial drug [e.g., N-2-thiazolyl nicotinamide (1948-52)], unlike those of the amide group but all of substituted forms endowed with antitubercular activity as triamino isonicotinic acid and its methyl ester were lacking in vitamin properties. Relatively little attention had been focused on the hydrazine fraction of INH. The benzalbenzenic hydrazide had displayed in vitro activity at a concentration of 10^{-6} M. The hydrazides of nicotinic acid and its derivatives had very limited (but structurally interesting) in vitro but were inactive in vivo. The fundamental mechanism of action of isoniazid is the inhibition of mycophenolic acid synthesis.
INH proved to be an almost ideal antitubercular drug. In addition to being fully selective, the drug has never been exceeded by other anti-TB drugs. It displayed marked clinical activity in various forms of tuberculosis after a few days of treatment, and could be given during pregnancy. Both the frequency and severity of its adverse effects (hepatic, peripheral neuropathy that could be managed with vitamin B6 supplementation, CNS effects, immune, allergic, and hematologic disturbances) were more than acceptable. Given its activity at the CNS level, INH therapy has been proposed for certain neurologic disorders.
INH was (and is) a drug fully deserving of the appellative 'magic bullet'.

A New Paradigm Of Endocrine Systems That Enables The Development Of Selective Hormonal Therapies

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The concept of hormones as "chemical messengers" involves implicitly a paradigm which can be expressed briefly as follows: a hormone has a single physiological function; whether produced *endogenously* or administered *exogenously*, a hormone elicits always the same responses, which are independent of any other factors. Many observations by different groups of investigators do not support such notions; different *endogenous* macropulses of a hormone elicit different responses and thus have different physiological functions that are carried out independently of each other in a coherent manner. This contrasts with the diverse, incoherent effects produced by the *exogenous* administration of a hormone. As different endogenous macropulses of a hormone may be elicited by many different hormones, some of us have proposed earlier that hormone macropulses are components of multisignal messages which produce selective, coherent responses. By monitoring macropulses of a hormone together with their known diverse elicitors, as well as with their known diverse responses, the compositions and effects of the different multisignal messages of which the hormone is a part can be identified. Analytical methods recently developed by which numerous variables can be monitored simultaneously, make such goals realístic. The therapeutic production of such multisignal messages would elicit selective hormonal effects, and would avoid the production of undesirable adverse effects of exogenous single hormone administrations.

Searching For A Tool To Improve The Anti-Doping Action: The Project AR.I.E.T.T.A. (Artificial Intelligence Evoking Target Testing In Antidoping)

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Background: Substances and methods used to increase the oxygen blood transport and the athlete's performance can be detected but the screening phase performed by International Federations remains a critical issue. The project AR.IE.T.T.A. aimed to develop a software able to analyze athletes' haematological and performance profile and to point out those reflecting an abnormal pattern.
Methods: 120 Athletes belonging to the International Biathlon Union gave their written informed consent to the study. The haematological and performance data, previously collected were used to develop the AR.I.E.T.T.A. software.
Results: The software includes the following sections: 1) Log-in 2) Data-Entry: data can be loaded, stored and grouped 3) Analysis: data can be analysed, validated scores calculated, parameters displayed simultaneously as statistics, table/graphs, individual or subpopulation profiles 4) Screening: an immediate evaluation of the risk score of the present sample and/or the athlete under study can be obtained. The risk score is calculated combining different parameters, absolute values and inter-intra-individual variations considered concurrently with different weights.
Conclusions: AR.I.E.T.T.A. software enables a quick evaluation of blood results, favouring surveillance programs and timely target testing controls on athletes by the International Federations. Future studies aiming to validate the risk score and to improve the diagnostic phase will enable an upgrade of the system.

Albendazole In The Treatment Of Hydatidosis Of The Muscles

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Background: Hydatid disease caused by the tapeworm Echinococcus granulosus is a worldwide problem especially in sheep and cattle raising countries. Muscle involvement is most commonly encountered as recurrence of previously treated disease or concurrently with primary lesions of the liver or lung. Furthermore, the rarity of muscle hydatidosis has unique implications in diagnosis and management.
Methods: We report here our observations on the usefulness of perioperative chemotherapy in surgical outcome in terms of morbidity and recurrence. We report on eight cases of primary echinococcus of the muscles presented in our clinic during a 10-year period.
Results: We have administered preoperative albendazole for one cycle of 28 days in 6 of our patients based on the size and appearance of the cyst. All patients underwent total pericystectomy without cyst rupture. We have not found any recurrences after minimum follow up of 12 months.
Conclusions: Muscle hydatidosis respond well to surgical intervention. Complete and intact removal of the cyst in muscular hydatidosis should be considered curative.

Enhanced Depot Vaccine Formulations, Vaccimax® And Depovax™, For Cancer And Pandemic Influenza Applications

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Background: The development of a vaccine that can induce a robust cellular and humoral antigen specific immune response after a single dose would be ideal for pandemic flu and cancer immunotherapy. IVT has developed two novel depot vaccine formulations, VacciMax® and DepoVax™, which induce enhanced immune responses when compared to current common treatments.
Methods: To detect humoral responses induced by the recombinant H5 antigen (Vietnam/04), mice were immunized once with IVT's vaccine formulations and once or twice with an appropriate control vaccine. Serum was collected at week 2, 3, 4 and monthly thereafter and titered using an H5 ELISA. The therapeutic efficacy of cacner vaccine formulations was tested in an established tumor challenge model. Mice bearing C3 tumors were vaccinated once with IVT's depot formulations containing the immunodominant CTL epitope from HPV 16 E7. Tumor growth and survival was monitored for 6 weeks. Antigen specific CTL activity was detected by IFN-γ ELISPOT in the lymph nodes of HLA-A2/H2-D (AAD) transgenic mice immunized with HLA-A2 restricted CTL epitopes formulated in IVT's depot vaccines.
Results: H5 formulated in our depot technology was able to raise a strong immune response within 18 days. At all time points tested, IVT depot formulation titers were superior to a single dose of the control alum vaccine and in the longer term superior or equal to the two dose alum vaccine. In the therapeutic C3 tumor challenge model, a depot formulation effectively eliminated C3 tumors after a single dose (100% tumor free mice) compared to non-vaccinated mice (0% tumor free, mean tumor size >2000 mm^3). An HLA-A2 peptide-based therapeutic cancer vaccine has been designed for Breast/ Ovarian/ Prostate cancers and antigen-specific immune responses were detected in AAD mice after a single dose.
Conclusions: 1) Single dose capability and 100% response rate of IVT's depot vaccines are significant in the context of a pandemic vaccine for which low initial responses and overall low individual response rates could lead to many deaths. 2) The multi-targeting strategy using tumor-specific peptides and potent cellular response induced by IVT's depot vaccines indicates a promising immunotherapy for cancer.

Comprehensive HIV/AIDS Care And Treatment As A Need For Quality Provision Of Antiretroviral Therapy: A Case Study From Dar Es Salaam Region, Tanzania

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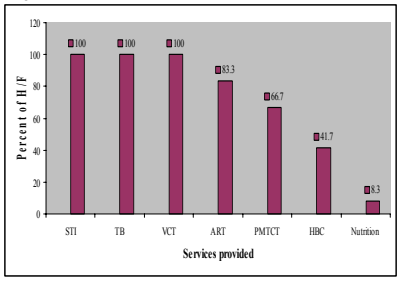
Background: The roll-out of Tanzania National HIV/AIDS care and treatment program began in October 2004, with a plan target to cover about 400,000 HIV infected Tanzanians in a period of five years. In delivering Antiretroviral Therapy (ART) a certain level of quality is recommended. The objective of this study was to determine the quality standards of health facilities in providing HIV/AIDS care and treatment in line with the Ministry of Health (MOH) stipulated guidelines.
Methods: A cross-sectional descriptive study was conducted to assess the quality standards in delivering ART in Dar es Salaam Region from May to July 2005. Ten health facilities (both public and private) already designated by MOH to provide ART, six of them since October 2004 (included purposively) and four since May 2005 (selected randomly). The other two facilities not designated were randomly picked and added. The checklist with the MOH required standards was used to assess the availability of equipments, staff, antiretrovirals, guidelines and adequacy of services provided.
Results: Regarding services provided, it was found that Comprehensive HIV/AIDS care and treatment was not fulfilled in all health facilities as recommended. More than half of the health facilities did not have Home Based Care (HBC) services. However, PASADA (Pastoral Activities and Services for people with AIDS in Dar es Salaam Archdiocese) with 14,000 patients, had a strong HBC with no patient lost to follow-up (The percentage of patient lost to follow- up ranged from 0% to 7.3%). Prevention of Mother to Child Transmission (PMTCT) services was found in two third of facilities. Although food support is included as an element of comprehensive HIV/AIDS care and treatment, only PASADA Voluntary Agency was providing nutrition services.
Figure: Percentage of health facilities with HIV care and treatment services



Conclusion: The advantage of comprehensive HIV/AIDS care and support services was shown by PASADA with the example of no patient lost to follow-up. The success shown by PASADA should be adopted by other health facilities for quality provision of ART.

Estrogen Metabolites In The Control Of Osteosarcoma

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Background: Osteosarcoma primarily affects children and young adults. Although a combination of surgery and chemotherapy has improved the survival rates, a specific therapy is yet to be determined. We have demonstrated that an estrogen metabolite, 2-methoxyestradiol (2-ME) is effective in killing osteosarcoma cells (bone cancer cells) and induces interferon gene expression. The goals of this study are: a) To determine the role of interferon-regulated RNA dependent protein kinase (PKR) in osteosarcoma apoptosis; and b) To develop a controlled drug delivery method for 2-ME in osteosarcoma cells.
Methods: 2-ME actions were investigated in MG63 human osteosarcoma cells by western blot analyses and cell proliferation assays. Oligo polyethyleneglycol fumarate (OPF) hydrogel was synthesized, mixed with 2-ME and cross linked using ultraviolet light. MG63 osteosarcoma cells were treated directly and with OPF hydrogel encapsulated 2-ME for a total period of 9 days.
Results: 2-ME treatment induced PKR kinase expression, activity and phosphorylation of the endogenous substrate, eukaryotic initiation factor-2□. Whereas, 17□-estradiol, 4-hydroxyestradiol and 16□-hydroxyestradiol did not induce cell death and had no effect on PKR protein. Ds RNA, an activator of PKR protein, increased cell death when osteosarcoma cells were co-treated with 2-ME. In contrast, PKR inhibitor 2-aminopurine blocked the 2-ME-induced cell death.
The hydrogel-mediated 2-ME delivery study shows that on day 3, cell survival was significantly less by direct treatment than in the hydrogels, indicating that there is no “burst” release of 2-ME from the hydrogels. However, hydrogels with 2-ME encapsulated showed extended effects as there was only 40% cell survival on day 9. This is significantly lower compared to direct treatment (p<0.0001).
Conclusions: Our results demonstrate that RNA-dependent protein kinase, PKR, through translational regulation contributes to proapoptotic action of 2-ME in osteosarcoma cells. In addition, OPF polymeric delivery system may prove to be very useful in sustained delivery of 2-ME and could be further explored for treating osteosarcoma and other cancers in vivo.

Driving Under Influence In France (2007) : Performance Of Accurate Emergency Forensic Procedures With Drug Urine Detection (Nal-Von Minden) Assessed By Blood GC/MS

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The SAM survey was conducted by the INRETS (France) to produce reliable epidemiological data concerning the role played by alcohol and drugs in fatal road accidents in France between 2001 and 2003. The high incidence (26%) of alcohol or drugs among the population of drivers from this study involved in fatal accidents has highlighted the importance for road safety of the consumption of these substances. By a February 2003 law, all drivers in France suspected to drive under the influence of a substance can undergo a urine test and, if that was not possible or the test proved positive, have a blood sample taken in order to test for drugs (cannabis, cocaine, opiates, amphetamines) either they are involved in any accident or not. The results are combined with the usual procedures of the police force, including the results of tests for alcohol levels. Drug screening through urinalysis is a widely accepted tool for rapid detection of potential drug use and therefore, a potentially useful method for detecting drug use in a variety of contexts such as the penal justice. Roadside drug testing was assessed in urine by means of Nal von Minden immuno-enzymatic drug urine analysis Ratisbonne tests with cut-offs as follows : 50 ng/ml for THC and metabolites, 300 ng/ml for opiates and cocaine metabolites, 1000 ng/ml for amphetamines. These levels are those defined by French road Code *(Décret n° 2001-751).* In order to obtain the most accurate results, emergency forensic physicians sampled and analysed urine of the drivers suspected of driving under influence by the police force, at any time, less than 30 minutes after police control. A whole number of 99 drivers, aged from 18 to 57 years, were examined and sampled in 2007 by the emergency forensic physicians of the Compiègne hospital (Picardy, France) to answer police or gendarmerie request.
Fifteen drug urine testing and breath alcohol analysis out of 99 were under cut-offs. Cannabis alone headed the list of illicit drugs detected, with a prevalence of 63.6 % (THC metabolites ≥ 50 ng/ml in urine); it was mostly present in the under-38s and especially the under-28s. In 10 other cases, urine drug analysis retrieved THC metabolites with another drug in 9 out of 99 (9.09%) and with two other in one case 1/99 (1,01%). Alcohol positive breath analysis was associated with THC positive urine drug testing in 3/99 cases (3,03%) and found alone in 7 other cases (7,07%). When urine Nal von minden test proved positive, suspected drivers had a blood sample taken by the required emergency forensic physician in order to test for drugs (cannabis, cocaine, opiates, amphetamines) within the 10 minutes following the test reading. Extraction and quantification of Delta(9)-tetrahydrocannabinol (THC) and 11-nor-9-carboxy-Delta(9)-THC (THC-COOH) and for the detection of 11-hydroxy-Delta(9)-THC (11-OH THC) in whole blood was realised by validated gas chromatography-mass spectrometry (GC-MS) technique. Same method was used for opiates, amphetamine(s) and cocaine. Urine drug testing results were compared with blood mass spectrometry results and demonstrated 100% sensitivities on any drug class. Specificities reached 100% for amphetamine(s), cocaine and opiates. In all cases a urine positive test for THC metabolites was correlated to a positive blood GC/MS result either for THC, 11-OH THC or THC-COOH. Since THC-COOH is not an active metabolite and since French law considered positive results in blood only if ≥ 1 ng/ml for THC and 11-OH THC, 76/84 results (90.4%) were considered positive both in blood and urine for active THC metabolites. Mean alcohol blood level was 1.19 g/l ± 0.37 [0.52-1.8] and 1.57 g/l when associated to cannabis. Cannabis was respectively associated to alcohol in 3, to cocaine in 1, to opiates in 3 and to amphetamines in 2 cases. Three substances were found in one driver under influence. Mean THC blood level measured by GC/MS technique was 6.29 ± 4.32 ng/ml [1.01-19.83] and mean 11-OH THC level 2.48 ± 1.14 ng/ml [1.11-5.06]. These levels are much higher than those required by French road Code (≥ 1 ng/ml for THC or 11-OH THC] to assess the road traffic offence. Moreover, mean THC-COOH level measured by GC/MS, linked to the frequency or concentration of cannabis use, reached 48.61 ± 40.62 ng/ml [4.24-221.15], which highlights the fact that most of the drivers sampled positive were regular users of cannabis or users of concentrated forms of cannabis.

Is Cannabidiol (CBD) An Accurate Marker For Cannabis Use? Analysis Of 2007-2008 Blood GC/MS Analysis From Drivers Under Influence

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Cannabinoids are the natural constituents of cannabis. The main of them are delta-9-tetrahydrocannabinol (Δ-9THC), psychoactive agent, cannabinol (CBN) and cannabidiol (CBD). A single active dose of 9THC is estimated on 520 mg. Δ-9THC is rapidly metabolised. It is hydroxylated to an active metabolite, 11-hydroxy-delta9-tetrahydro-cannabinol (11-OH-THC), then oxidised to an inactive 11-nor-9-carboxy-delta9-tetrahydrocannabinol (THC-COOH), which is conjugated with glucuronic acid and predominantly excreted in the urine. Adverse effects such as anxiety, changes in sensory perception, impairment of memory and psychomotor performance, increased heart rate and changed blood pressure which may have serious consequences are common after a dose is taken that exceeds an individually variable threshold. The maximum effect persists for 4-6 h after administration despite of very low Δ-9 THC blood concentrations. Δ-9 THC plasma concentration declined to values of 2-3 ng/ml during 3-4 h after smoking. Such a low concentration of the active compound justifies the use of sensitive analytical methods for detection and determination of Δ-9 THC and its metabolites. The most effective techniques for Δ-9 THC and related compounds determination in biological material are chromatographic ones (gas and liquid) with mass spectrometric detection and different ionization modes. Δ-9 THC and its two metabolites (11-OH-THC and THC-COOH) are present in blood. Presence of delta(9)-tetrahydrocannabinol (THC), the major psychoactive constituent of cannabis and its various preparations, and its active metabolite 11-hydroxy-delta9-tetrahydro-cannabinol (11-OH-THC) is verified by analysis of blood by gas chromatography-mass spectrometry (GC-MS) of individuals apprehended for driving under the influence of drugs if their urine screened by enzyme immunoassay method was THC positive. Extraction and quantification of active métabolites THC and 11-OH THC by GC/MS is currently used for samples collected by emergency forensic physicians from individuals suspected of driving under influence, as soon as possible after the police control and drug intake. Following Emergency Forensic Medicine department guidelines, most of the drivers, if positive in urine, were sampled for blood at any time, less than 45 minutes after police control. Hybrid varieties of cannabis are known to contain a higher potency through higher than usual levels of the active ingredient THC as "skunk" or « madweed », an hybrid plant originating from Afghanistan, Morocco, Holland and Thailand, specifically bred to produce a very high level of THC. Where the standard cannabis can be expected to have a THC content of about 1% to 5%, skunk has been known to contain as much as 30% and skunk users have reported experiencing intense paranoia or cardiovascular complications. A study of UK street cannabis published in the *Journal of Forensic Sciences* suggested that cannabis resin has the average highest rates of cannabidiol, while 'skunk' and imported herbal cannabis (weed) have the lowest. The very high THC levels measured in blood samples sent to a reference laboratory attracted our attention. Extraction and quantification of cannabidiol (CBD), a natural constituent of cannabis, was realised by GC/MS, simultaneously to those of THC and 11-OH THC to verify some hypothesis concerning for example the time of cannabis intake, and the type of cannabis used. A serial of 160 samples collected in 2007 in people positive in urine for cannabis abuse was analysed. On one hand, mean level of THC was 4.64 ± 6.02 ng/ml [0.1-48.4], mean level of 11-OH THC was 2.46 ± 2.64 ng/ml [0.09-15.03]. On the other hand, mean level of CBD was 3.33 ± 4.37 ng/ml [0.06-36.29]. Mean difference between THC and CND dosages is 1.31 ng/ml, with a mean difference value of 15.1% ± 40%. Mean difference between THC and 11-OH THC dosages is 2.18 ng/ml, with a mean difference value of 19.1% ± 109%. Correlation curves give interesting results between CBD and THC and discrepancies mainly seem related to the use of skunk or weed, known to be CBD free. We believe that cannabidiol may be dosed as an accurate marker of type and quantity of cannabis used and may bring some information about some deaths by overdose of cannabis which are more and more frequently encountered in forensic medicine.

Anti-Ige Therapy In The Management Of Asthma: Present Use And Possible Future Applications

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The treatment of asthma has evolved over the past 50 years and asthma is now commonly viewed as an inflammatory disorder of the airways. This has led to a shift in treatments from bronchodilators to anti-inflammatory agents. Despite the availability of effective therapies, there continues to be a need for additive therapies to improve asthma control. Inhaled corticosteroids are widely accepted as first-line therapy for almost all patients with persistent asthma. For patients with more severe asthma, additional agents are often needed to achieve asthma control. The development of omalizumab, a monoclonal antibody against IgE has led to improvement in asthma control and subsequently in the quality of life for many patients. It is widely accepted that approximately 60% of asthma is IgE mediated, with even higher numbers in children, and that therapy directed against IgE can result in significant reductions in asthma exacerbations. This would translate to a reduction in urgent care visits, including emergency department visits and hospitalizations. The recent guidelines for the management of asthma (*GINA* [Global Initiative for Asthma] and *NHLBI* [National Heart Lung and Blood Institute]) now stress the pivotal role of omalizumab in the treatment algorithms for these patients.

Omalizumab, a monoclonal antibody against IgE was introduced into the United States in 2003 and has become part of the therapeutic armamentarium to improve asthma control. Three pivotal studies demonstrated efficacy in patients previously treated with inhaled corticosteroids alone and subsequently at least two studies have shown efficacy in patients treated with broader regimens that have included long-acting beta agonists and leukotriene modifiers. In addition to resulting in reductions of free IgE, the use of omalizumab has also resulted in down-regulation of the high-affinity IgE receptor and in the ability of dendritic cells to process allergens. At present, omalizumab is approved for the treatment of “difficult to treat” asthma in those age 12 years of age and older. It is anticipated that pediatric use will soon be recognized and perhaps future uses will include the administration of this compound at an earlier stage in an effort to act as a true disease modifier.

Mannose-Binding Lectin In The Defence Against Genital Candida Infections

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Background: Mannose-binding lectin (MBL) is active in the innate immune defence against genital infections. MBL binds to *C. albicans* and is considered to protect against vulvovaginal candidiasis (VVC) and such recurrent infections (RVVC). RVVC is not a chronic infection but characterized by repeated attacks. RVVC cases are often candida culture-negative in spite of symptoms and signs of vaginitis in between the attacks.
Material and Methods: Twenty-nine women with a history of RVVC were investigated, who had all consulted the Gyneaecological Department at the University Hospital, Lund. The comparison group consisted of 30 women who were staff at the Department. All patients reported to a standardized history form and were subjected to a speculum investigation.
Cultures of vulvar and vaginal samples collected from the lateral vaginal wall and the posterior fornix were grown on Chromagar, which allowed speciation of any candida isolate. Serum levels of MBL were determined by a sandwitch time-resolved immunofluorometric assay, using anti-MBL coated microtiter wells containing, samples, which were washed and incubated with biotinylated anti-MBL followed by europium-labelled streptavidin and measured by time-resolved flurometry.
Results: The serum levels of MBL in the RVVC group, age 21-58 (mean 31) years, ranged from <10 to 5892 ng/mL (mean 1590 ng/mL). The corresponding figures for the comparison group, age 21-64 (mean 41) years, were <10 to 2987 ng/mL (mean 920 ng/mL). The difference was significant (p=0.006). The levels were higher in culture-positive (44,8%) than culture-negative RVVC patients (p=0,02). *Candida albicans* made up all, but one (*C. glabrata)* of the isolates. RVVC who were candida-negative when consulting had MBL levels comparable to the controls. There was no difference in MBL levels between women of different age (10 years ranges).
Conclusions: MBL levels were higher in women with a history of RVVC than in the controls as well as higher in those who at sampling occasion were candida-positive compared with candida-negative women. MBL may have a therapeutic effect in cases of recurrent gentital candida infections.

Prospective, Structured Data Collection For Recombinant FVIIa: The STER Experience On 55 Surgical Interventions In Patients With Congenital FVII Deficiency

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Background: Being bleeding a major complication of surgery, its control is essential for the outcome of any operation. This is even more important when surgery is performed in patients with Congenital Bleeding Disorders (CBDs). CBDs are usually identified by a positive bleeding history, but these patients may undergo a surgical operation undiagnosed, usually because of a negative history. The severity of bleeding in the surgical scenario depends on the type of intervention, the severity of the CBD, the presence of co-morbidities and the efficacy of the replacement therapy. In FVII deficiency, a prevalence of 23.5% of peri- and post-operative bleeds was reported by us, together with not a negligible prevalence of thromboses (3.5% of patients), spontaneous or treatment-related. The occurrence of an inhibitor to the missing factor is also a severe complication of treatment. A structured evaluation of a large number and variety of surgical cases, is an excellent method to perform the pharmaco-vigilance of replacement therapies.
Methods: Within the frame of a multicentre, prospective, observational study, using an online registry, we have evaluated 55 surgical interventions (27 "major", 28 "minor") treated with recombinant FVIIa (n=46) or a plasma-derived FVII concentrate (n=9), to protect 48 different patients with a FVII deficiency (18 severe & moderate and 30 mild) from surgery-related bleeding.
Results: Mean daily dosages ranged from 1.2 to 87 µg/Kg/bw for rFVIIa and from 6 to 34 IU/Kg/bw for the pdFVII concentrate. Replacement therapy was carried out for a median of 9 d. (1-37) for the "major" and 4 d. (1-16) for the "minor" surgeries. A mild bleeding event occurred (2nd d) in a patient treated with rFVIIa. An antibody to FVII (max titre 10 BU) appeared in a patient treated with rFVIIa who, nonetheless, had received plasma-derived products, previously. No thrombotic events occurred.
Conclusions: Both kind of replacement therapies proved safe and efficacious for the treatment of FVII deficiency. Major side effects, clearly related to either the pdFVII concentrate of rFVIIa, did not occur. The wide range of replacement therapy duration and dosages outlines the need for a consensus focused on the identification of optimal replacement therapy schedules in both minor or major surgeries.

The Role OF Heat Shock Proteins IN Oral Cancer: A Possible New Target IN Antineoplastic Therapy?

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Background: Heat shock proteins (HSPs) are a group of highly immunogenic proteins with an exceptional degree of conservation. They are expressed or increased in response to various biological stresses, while their best known physiological role is to act as molecular chaperones.
Methods: Immunohistochemistry staining was conducted to study HSP70 expression in 50 paraffinized tissue samples; 30 oral squamous cell carcinomas, 10 leukoplakias with dysplasia) and 10 samples from normal oral tissue.
Results: Our preliminary results showed that in squamous cell carcinoma, the cytoplasm and the cell membrane of the tumor cells was often positive for HSP70. Dysplastic lesions were positive to a lesser extent for HSP70. Samples from normal oral tissue were negative for HSP70.
Conclusions: It is concluded that HSP70 immunoexpression could be a marker for the presence of epithelial dysplasia or epithelial malignant transformation. Our results agree with certain of the literature. Overexpression of HSPs has been observed in certain cancers and their physiopathologic activity in tumorigenesis is related to cell growth and differentiation. It is known that members of the heat shock protein family (i.e. HSP70 and HSP90) are often associated with cell-cycle-related proteins including p53, Cdk4, c-myc, pRb and p27. Along with their intracellular chaperoning function, HSPs have been found to play key roles in cancer immunity by chaperoning tumor-derived peptides to major histocompatibility complex (MHC) class I molecules to elicit an anticancer immune response mediated by cytotoxic T lymphocytes. It has been demonstrated that physical as well as chemical stress increases the amount of cytoplasmic and plasma membrane-bound Hsp70, while normal cells even after exposure to stress fail to express HSP70 on their plasma membrane. Additionally, a tumor-selective cytoplasmic - membrane expression has been found to correlate with an increased sensitivity to the lytic activity mediated by natural killer cells. Taking all these into account we can postulate that HSP70 in the future can be an ideal target structure for a molecular-based anticancer immunotherapy.

Activation Of Exploratory Behavior In Senescence Accelerated OXYS Rats By Stimulation Of Cell-Mediated Immune Response With BCG Vaccine

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Background: Changes in cognitive and emotional spheres are typical of aging. Their prevention is an obligatory condition providing active longevity of elderly people. We demonstrated that senescence-accelerated OXYS rats constitute appropriate model for studying ageing processes. Changes in exploratory activity and the degree of anxiety in OXYS rats are significantly related to activity of cellular immune reactions. The aim of our study was to clear out whether it was possible to modify the behavior of OXYS rats by stimulating cellular immune reactions.
Methods: This study included 40 3-month-old male OXYS and 10 Wistar rats. All animals were tested in an "open field", after which some animals were intraperitoneally injected with dry tuberculous BCG vaccine (Allergen Firm) in doses of 50 or 600 µg/kg in 0.5 ml RPMI-1640. Controls were injected with the same volume of the solvent (RPMI-1640) under similar conditions. Two weeks after injection the parameters of exploratory behavior, cellular immune reactions were repeatedly evaluated.
Results: During the first open-field testing horizontal and vertical motor activities of OXYS rats were, as expected, markedly lower than of Wistar rats. High anxiety characteristic of OXYS rats manifested in a 3-fold lower number of grooming reactions in comparison with repeatedly tested Wistar rats and 5-fold greater number of boluses. Stimulation of the cellular component of the immune response by injection of BCG vaccine significantly modified horizontal motor activity of animals. The effect was dose-dependent and peaked after injection of 600 µg/kg vaccine: in this case horizontal motor activity increased 3-fold and did not differ from that of Wistar rats. Animals receiving the maximum dose of the vaccine did not differ from Wistar rats also by the parameters of vertical motor activity and number of grooming reactions.
Conclusions: Stimulation of cell-mediated immune response with BCG vaccine caused a dose-dependent activation of exploratory behavior in senescence accelerated OXYS rats. The possibility of correction of behavioral changes associated with early aging of OXYS rats by stimulation of cellular immune reactions is a direct proof of the involvement of neuroimmune relationships in the process of aging.
Authors' disclosure statement: OXYS rats bred at Institute of Cytology and Genetics (Novosibirsk, Russia) is an adequate model of accelerated aging and aging-related neurodegenerative processes. OXYS rats show shortened lifespan coupled with several geriatric disorders such as early cataract, macular dystrophy, hypertension, suppression of the cell component of the immune system, as well as changes in cognitive and emotional spheres (increased anxiety, disturbances in associative learning, decreased exploratory activity in the open field test) develop in OXYS rats by the age of 3 months.

Effective Drugs In Psychiatry: Magic Bullets Or Hand Grenades?

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Background: Neuropharmacological research has improved understanding of the molecular basis of psychiatric diseases including depression and schizophrenia. As a result, drug development has focused on identifying compounds that selectively influence the relevant molecular targets. Although selective agents may have tolerability advantages, it is unclear whether increases in selectivity have enhanced or compromised efficacy in the treatment of depression and schizophrenia.
Methods: Literature was reviewed to compare relative efficacy among antidepressants and antipsychotics with particular focus on the neuropharmacological profiles of these drugs. Selective serotonin reuptake inhibitors (SSRIs) were compared to broader antidepressants affecting serotonin and norepinephrine such as serotonin/norepinephrine reuptake inhibitors (SNRIs), monoamine oxidase inhibitors, and mirtazapine. Additionally, the dopamine-specific antipsychotic haloperidol was compared to agents with broader receptor-binding characteristics including clozapine and olanzapine, and available comparative efficacy data among antipsychotics in general were evaluated with attention to the receptor selectivities of the various drugs.
Results: There are some indications of a statistically-but not necessarily clinically- significant advantage in antidepressant efficacy advantage of drugs enhancing neurotransmission of both serotonin and norepinephrine compared to SSRIs. There is no consistent evidence that SSRIs or SNRIs are superior in efficacy to older non-selective antidepressants. Newer non-selective antidepressant agents under development such as the triple uptake inhibitors hold promise. Similarly, studies support greater antipsychotic efficacy with the broad spectrum agents such as clozapine compared to haloperidol in the treatment of schizophrenia.
Conclusions: 1) Broad spectrum antidepressants and antipsychotics have demonstrated comparable and sometimes superior efficacy compared to more selective agents. 2) The advantages of selective agents over the broad spectrum agents have been in tolerability as opposed to efficacy. 3) Since multiple neurotransmitter systems are affected in most psychiatric disorders, future drug development efforts should focus on developing broad spectrum agents that optimize efficacy without compromising tolerabilty.
Authors' disclosure statement:
Dr. Marks has received research support from Merck and National Institutes of Health and is on the speaker's bureau of Eli Lilly and Company and Pfizer. Dr. Patkar is a consultant for Bristol-Myers Squibb, GlaxoSmithKline, and Reckitt Benckiser; is on the speaker's bureau of Bristol-Myers Squibb, GlaxoSmithKline, and Reckitt Benckiser; has received research support from National Institutes of Health, AstraZeneca, Bristol-Myers Squibb, Forest, GlaxoSmithKline, Janssen, McNeil Consumer and Specialty Inc, Organon, Jazz Pharmaceuticals, and Pfizer.

Can We Make Eucaryotic Cells Resistant To Antibiotics? Correlation Between Multidrug Resistance-Associated Protein (Mrp) Efflux Pump Expression And Fluoroquinolones Accumulation In J774 Macrophages

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Background: Over-expression of multidrug transporters is a well-known mechanism of resistance to anticancer agents in eucaryotic cells, and to antibiotics in prokaryotic cells. In vitro, their overexpression can be obtained by prolonged exposure to drug substrates. We have examined here whether chronical exposure to fluoroquinolone antibiotics could trigger a phenotype of resistance in eucaryotic cells as well, based on our previous observation that ciprofloxacin (CIP) is subject for an active efflux by an Mrp–like (Multidrug resistance-related Proteins) transporter in J774 macrophages (MΦ), which decreases its accumulation and activity against intracellular bacteria, while moxifloxacin (MXF) was not affected (AAC 2005, 49:2429-2437).
Methods: J774 MΦ were exposed for several months to increasing concentrations in either ciprofloxacin or moxifloxacin (from 17 to 68 mg/L). CIP and MXF accumulation was measured by fluorimetry in the resulting cell lines as compared to wild-type MΦ. Mrp expression was evaluated by real-time PCR and Western-blot.
Results: As compared to wild-type MΦ, which accumulate CIP about 4-fold and extrude it with a $t_{1/2}$ ~ 1.2 min, cells exposed to CIP showed a markedly decreased accumulation of CIP (< 1-fold) related to a faster efflux ($t_{1/2}$ < 0.1 min), while cells exposed to MXF showed a higher accumulation of CIP (15-fold) and a slower efflux ($t_{1/2}$ ~ 2.6 min). MXF accumulation was high (15-fold) and similar in all cell types.
Analysis at the mRNA and protein levels revealed an overexpression of Mrp2 and Mrp4 in MΦ exposed to CIP and a reduction of Mrp4 expression in MΦ exposed to MXF.
Conclusions: Exposure of J774 MΦ to the Mrp substrate CIP selects for a resistant phenotype characterized by the overexpression of two Mrp transporters. Exposure of the same cells to the non-substrate MXF selects for an 'anti'-resistant phenotype, with reduction in the expression of Mrp4. These data suggest that fluoroquinolones can differentially affect the expression of Mrp transporters in J774 MΦ, illustrating the complexity of the interactions between closely-related drugs and transporters.

Dual Inhibitors Targeting Matrix Metalloproteinases And Carbonic Anhydrases As Potential Anticancer Drugs

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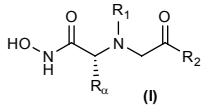
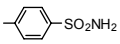
Background: Matrix metalloproteinases (MMPs) and carbonic anhydrases (CAs) are two classes of zinc-dependent enzymes with different roles and catalytic targets, such as the degradation of most of the extracellular matrix (ECM) proteins, and the regulation of the CO_2/HCO_3^- equilibrium in the cells, respectively. Both families have isoforms which have been proved to be involved in several stages of carcinogenic processes, and so the selective inhibition of these enzymes might be of interest in cancer therapy.
Methods: We report herein the design, synthesis and *in vitro* evaluation of a series of compounds possessing the iminodiacetic acid as the main backbone and two functional groups attached, namely the hydroxamic and the arylsulfonamide ($ArSO_2NH_2$) moieties, to enable the inhibition of MMPs and CAs, respectively. Docking studies were also performed in order to investigate the binding interactions formed between ligand-protein.
Results: Considering the general formula (I) of the iminodiacetic (IDA) mono-hydroxamate derivatives, several substituent groups were tested. The introduction of the 4-sulfamoylphenylethyl (SPE) moiety as R_2 group lead to a general increase of activity with most MMPs but, more significantly, endowed with the ability of inhibiting CAs. The apolar isopropyl group in □-position to the hydroxamic group also reflected an increase of inhibitory activity with both MMPs and CAs.



(I)

SPE = -CH_2CH_2-  -SO_2NH_2

(R_1 = -SO_2PhOPh)

Comp.	$R_\square$	R_2	IC_{50} (nM) MMP-1	IC_{50} (nM) MMP-2	K_I (nM) CA II	K_I (nM) CA IX
2	H	OH	1.53×10^3	1.2	-	-
16	H	SPE	143	1.57	65	12
20	*i*-Pr	SPE	7.8	0.35	4.2	3.3

Docking studies revealed interaction of the SPE group with conserved residues of S_2'-S_3' subsites of the MMPs, this being the reason for the high affinity and low selectivity of these compounds with this family of metalloenzymes.
Conclusions: This work demonstrated that the introduction of the 4-sulfamoylphenylethyl group in the IDA scaffold maintains the activity on MMPs, providing a good activity also on some CAs. This finding opens the way to further studies directed to the identification of dual inhibitors selective for those enzymes implicated in cancer, such as MMP-2 and CA IX.

Success Story Of The First Regulatory Approval Of Safety Biomarkers, Part I: From Identification To Biological Qualification

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Background: In the context of the Critical Path Initiative, a project on the validation and qualification of new renal safety biomarkers has found a successful closure.
Methods: The project focused on rat nephrotoxicity, whereby 8 known nephrotoxicants and 2 known hepatotoxicants were administered at different dose levels to assess diverse modes and severity grades of toxicity. To enrich the set of functional markers (serum creatinine and blood urea nitrogen) used for the last hundred years, gene expression profiling was performed in the kidney and correlated to the histopathology.
Results: Transcriptomics allowed the identification of 15 promising new injury biomarkers covering toxicity in proximal tubules, distal tubules, and glomerules. These 15 biomarkers were then used to monitor changes of their protein levels in kidney, blood and urine.
This project also allowed the establishment and testing of the implementation of an early safety biomarker validation process. The first step of the process was the discovery phase and selection of exploratory biomarkers, model compounds and the definition of the animal model. Then, appropriate analytical methods (multiplexed immunoassays and RT-PCR) were developed and validated. The in-life phase started with a dose-range finding study followed by the main validation studies comprising of 960 animals in total. In these multiple models of kidney injury, the sensitivity and specificity of the new markers were assessed and compared to the "gold standards" serum creatinine and blood urea nitrogen.
In addition, a gene expression atlas across multiple organs in baseline and treated animals was compiled for the best performing markers.
Conclusions: A subset of these markers demonstrated higher diagnostic performance than serum creatinine and blood urea nitrogen, especially for low-grade tubular and glomerular injury. These data were submitted to the FDA and EMEA as first Voluntary eXploratory Data Submission (VXDS) and subsequently 5 urinary biomarkers were approved for use as biomarker to monitor renal injury in pre-clinical studies.

Enerceutical Mediated Activation Of The Alternative Cellular Energy Pathway In The Therapy Of Infectious Diseases

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Background: Stealth adaptation of viruses refers to the loss of the relatively few virus antigens that are normally targeted by the cellular immune system. Consequently, these viruses evade effective recognition by the cellular immune system. Stealth adapted viruses are postulated to be a major cause of human illnesses, especially those with prominent neuropsychiatric features, including autism in children and depression/cognitive disorders in adults. A non-immunological, auxiliary defense mechanism can repair the cytopathic effect (CPE) caused by stealth adapted viruses in tissue cultures. The repair is mediated by particulate, pigmented materials that are typically fluorescent, occasionally magnetic and can show both electron donating and water splitting capacities. Ultraviolet (UV) light evoked fluorescence can commonly be enhanced using various dyes, including neutral red. The materials seemingly provide a non-mitochondria source of cellular energy. Alternative cellular energy (ACE) pigments are detectable in tissues and body fluids of patients with various illnesses. Comparable materials, termed Enerceuticals, are being formulated for potential clinical use in illnesses caused by both stealth adapted and conventional viruses.
Methods: In an ongoing study in patients with autism, paper towels moistened with a particular Enerceutical preparation and neutral red dye, are layered onto a polyethylene sheet that covers parts of the body. The paper towels are rendered fluorescent using UV-A illumination. The patients are observed for skin fluorescence occurring elsewhere on the body and for post-treatment signs of clinical improvement. A similar approach is being used in the therapy of patients with recurrent herpes simplex virus (HSV) infections and with HZV induced post-herpetic neuralgia.
Results: Major clinical improvements, described and updated regularly at www.iminhere.ca, are occurring in autistic patients following 2-5 daily, 30-60 minute sessions using the above protocol. Single therapies are also achieving expedited healing of active HSV and HZV lesions, along with a marked reduction in HSV recurrences and in the severity of HZV associated neuralgia.
Conclusion: Activation of the ACE pathway can provide an effective means of treating illnesses due to both stealth adapted and conventional virus infections.

How To Implement Pharmaceutical Care In The Curriculum?: The Cuban Pharmacy Education Experiences

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Background: Pharmacy schools across Cuba have been charged to ensure their students are adequately skilled in the principles and practices of pharmaceutical care. Despite this mandate, a large percentage of students experience insufficient opportunities to practice the activities, tasks and processes essential to pharmaceutical care. This paper presents a point of view about how pharmaceutical care should be incorporated in the curricula for improving the confidence and skills of pharmacists responding to pharmaceutical care practice taking into consideration the ethical dimension of this concept. At the same time, some ideas about this topic are presented, taking as reference, the Cuban experience in pharmaceutical care education, supported in the worldwide recognition of the Cuban Higher Education.
Methods: theorical research methods were applied, such as analysis and synthesis, and statistical method.
Results: The theoretical contribution of this research lies in offering a didactic model to implement pharmaceutical care in to the curricula, the knowledge, abilities and professional values are considered as a system, to provide all the principles and practices components for the pharmaceutical care in the pharmacy curriculum.
Conclusions: Many methods to be used to teach student to provide pharmaceutical care, but it is important to understand that the clinical pharmacy method as logical expression of pharmaceutical care process should be taught; taking into account that, the professional method acquires during the teaching – learning process a greater importance than answering a specific problem. The scientific facts and data learnt today can become obsolete or even not be accepted in a near future. On the contrary, those pharmacists who can identify and solve their patient's drugs - related - problems by applying a reasonable method will be able to adjust themselves to the continuous and speedy evolution of scientific knowledge so as to scientifically contribute to a better health and their patient's quality of life.

New Horizons In Respiratory Allergy Therapy And "Magic Bullets": Could It Be Possible To Include Antiprotozoal Drugs?

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Background: The allergic diseases that affect the respiratory pathways have reached epidemic proportions in recent decades. For many years now, a close relationship has been established between these diseases and products corresponding to certain arthropods such as mites and cockroaches. Although it is ten years since the presence of certain protozoa in the sputa of asthma patients was first described, observation of the existence of protozoa in the intestinal extracts of dust mites, as well as of other protozoan forms in the intestine of cockroaches, has recently led us to consider a possible etiopathogenic role in the development of respiratory allergy. This role may be reinforced if we bear in mind that the inhalation of faecal particles of such arthropods may introduce pathogens into the respiratory pathways. This suggests to us that the use of antiprotozoal drugs may be effective in the treatment of diseases such as bronchial asthma and allergic rhinitis.
Methods: To review the publications available in different fields of medicine that refers uncommon and unknown kinds of protozoa that may affect the human airways.The literature review was identified trough electronic data bases such as MEDLINE, EMBASE, and the COCHRANE DATABASE of SYSTEMATIC REVIEWS. Peer-reviewed publications in English, French, and Spanish language, and English-language abstracts of non-English papers, identified in our research, were included.
Results: Uncommon multiflagellated protozoa belonging principally to Phylum Sarcomastigophora, Order Hypermastigida, and observed in intestinal extracts of mites and cockroaches, also have been found in human respiratory airways secretions, especially in patients with respiratory allergy (bronchial asthma and allergic rhinitis) and/or immunosuppression status (AIDS, transplants, cancer, etc.).
Conclusions: It is evident that, despite great efforts and new therapeutic approaches, allergic respiratory diseases continue to be on the increase. Until now, only the possible role of certain microorganisms (viruses, bacteria and fungi) has been taken into account in the development of these diseases. The existence of uncommon protozoa related with arthropods such as dust mites and cockroaches may open up new etiopathogenic, therapeutic and preventive perspectives.
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Optimizing A Therapeutic With Nsaids: Intelligent Design For Delivery Systems

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Background: There is a wide approach to modulate drug release with the objective of optimizing therapy. The type of drug will define the type of release to achieve. The NSAIDs are used in anti-inflammatory diseases, so it would be desirable to develop a quick/slow delivery system to alleviate rapidly the painful symptoms and to avoid repeated administrations. Aims: 1) To develop biphasic delivery systems based on compressed or encapsulated mini-tablets 2) To study the dosage regimen flexibility.
Methods: Prolonged-release component: The mini-tablets contained either HPMC or EC as controlling agents and ibuprofen as a model drug. Mini-tablets, 12 mg and 2.5 mm, were prepared by direct compression in an instrumented mechanical press machine. Fast-release component: The filling of the void spaces between mini-tablets was formulated to produce an immediate release. The composition was identical for all formulations and contained the immediate release drug dose, microcrystalline cellulose and sodium croscarmellose. Compressed mini-tablets systems: The die of the tabletting machine was progressively filled with the weighed amounts of the fast release component and the mini-tablets prior to compression. Tablets were prepared by direct compression. Encapsulated mini-tablets systems: These systems were prepared by encapsulating the weighed amounts of the fast release component and mini-tablets in a hard capsule.
The dosage regimen flexibility was studied by combination of a different number of mini-tablets (prolonged release component) and a different dose of the drug (fast release component).
Results: The biphasic delivery systems were characterized by an initial rapid release, corresponding to the drug release contained in the powder component, followed by a period of slow release, corresponding to the drug release of mini-tablets. The release profile was dependent on the number and/or composition of subunits, making up the drug sustained dose. After the disintegration of the biphasic systems, the HPMC subunits were able to release a second dose fraction in a prolonged time (≈ 7h) at a constant rate and with an identical dissolution profile to the original mini-tablets. In the case of biphasic EC mini-tablets systems, the releasing of fast component disturbed the drug diffusion mechanism.
Conclusions: 1) Biphasic quick/slow preparations of ibuprofen were developed by compressing or encapsulating a combination of powder and mini-tablets. 2) The proposed biphasic delivery devices show flexibility in the modulation of the delivery program.

Biomedical Application Of Electron Spin Resonance (ESR) Spectroscopy Using Blood-Brain-Barrier (BBB) Permeable Nitroxyl Spin Probe

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Background: Oxidative stress induced by reactive oxygen species (ROS) are associated with the alterations under pathophysiological conditions, and particularly in brain ischemia, brain tumor, and neurodegenerative diseases. Therefore, we need to understand the physiological and pathophysiological role of oxidative stress induced by ROS in the brain.
Methods: This study examined two nitroxyl compounds, BBB-permeable 3-methoxycarbonyl-2,2,5,5-tetramethyl-pyrrolidine-1-oxyl (MC-PROXYL) and BBB-impermeable carbamoyl-PROXYL, as spin probes for evalution oxidative stress in the brain using by *in vivo* ESR or ESR imaging technique. Preliminary comparisons were made by *in vivo* ESR or ESR imaging of the heads of live mice and isolated rat brains using MC-PROXYL and the carbamoyl-PROXYL. These methods were also applied for the *in vivo* ESR or ESR imaging of isolated brains from spontaneously hypertensive rat (SHR) and stroke-prone SHR (SHRSP), which were well known high oxidative rodent model.
Results: The results showed that MC-PROXYL, but not carbamoyl-PROXYL, was widely distributed in the brain. The rapid decay of 2D ESR images of MC-PROXYL in isolated SHR-brain and SHRSP-brain was observed, compared to normotensive Wistar-Kyoto rats (WKYs), using the ESR imaging system. Furthermore, we provided evidence that the decay rate of MC-PROXYL in the head region was faster in live SHRs and SHRSPs than in live WKYs, by using *in vivo* ESR non-invasively. Taken together, the high oxidative stress sustained by ROS in SHR and SHRSPs may cause the alteration of MC-PROXYL metabolism in the brain.
Conclusions: Our results suggest that *in vivo* ESR could be applied to the assessment of antioxidant effects on oxidative stress in the brain in rodent disease models, such as the SHR and SHRSP. Further advances in the instrumentation of ESR imaging and would make this technology even more promising for the non-invasive diagnosis of oxidative stress induced-brain diseases *in vivo*. Furthermore, after screening test of drugs or foods using *in vivo* ESR technique, we'll be able to develop and find drugs or foods with novel antioxidant property in the near future.

Nobel Prize Winners In Medicine And Physiology

MAŠIĆ I

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The Nobel Prize in Physiology or Medicine is awarded once a year by the Swedish Karolinska Institute. It is one of the five Nobel Prizes established by the will of Alfred Nobel in 1895, awarded for outstanding contributions in physics, chemistry, literature, peace, and physiology or medicine since 1901.
The first Nobel Prize in Physiology or Medicine was awarded in 1901 to Emil Adolf von Behring, a German, "for his work on serum therapy, especially its application against diphtheria, by which he has opened a new road in the domain of medical science and thereby placed in the hands of the physician a victorious weapon against illness and deaths." This award is administered by the Nobel Foundation and widely regarded as the most prestigious award that a scientist can receive in these fields.
It is presented in Stockholm at an annual ceremony on December 10, the anniversary of Nobel's death. "The highlight of the Nobel Prize Award Ceremony in Stockholm is when each Nobel Laureate steps forward to receive the prize from the hands of His Majesty the King of Sweden. ... Under the eyes of a watching world, the Nobel Laureate receives three things: a diploma, a medal and a document confirming the prize amount" ("What the Nobel Laureates Receive"). In 2007 the Nobel Prize in Physiology or Medicine was awarded to Mario Capecchi (of Italy), Sir Martin Evans (of the United Kingdom), and Oliver Smithies (of the United Kingdom and the United States), "for their discoveries for introducing specific gene modifications in mice by the use of embryonic stem cells"; they share the prize amount of 10,000,000 SEK (slightly more than €1 million, or US$1.4 million). The front side of "The medal of the Nobel Assembly at the Karolinska Institute" provides the same profile of Alfred Nobel depicted on the medals for Physics, Chemistry, and Literature; its reverse side "represents the Genius of Medicine holding an open book in her lap, collecting the water pouring out from a rock in order to quench a sick girl's thirst" ("The Nobel Prize Medals").

Effect Of Formulations On Clopidogrel Bioactivity And Bioavailability In Vivo

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Clopidogrel hydrogen sulphate is antiplatelet agent approved for use in secondary prevention of heart attacks and stroke. Although millions of cardiac patients are benefiting from Clopidogrel treatment, however, nearly 5% of the patients are Clopidogrel "nonresponsive".
The pharmacokinetics parameters of Clopidogrel, under fasting conditions, are conflicting as to the maximum concentration achieved following the dose (Cmax) with a range of 1.2 ng/ml to 9 ng/ml and a time to maximum concentration (Tmax) of 1 hour to 2.5 hours.
These results are more controversial under feed conditions with studies showing no effect of food on the pharmacokinetics of Clopidogrel in Caucasians while studies in East Indian population demonstrated a significant influence of food. In these studies Tmax increased from 2.5 hours to 5 hours and the Cmax was increased by 5 folds. Moreover, there is a direct effect of genetics on the absorption and thereby active metabolite formation which are diminished by P-gp-mediated efflux and are influenced by the MDR1 C3435T genotype. We have performed a comparative study on the bioavailability and correlated that with in vivo bioactivity, as measured by inhibition of platelet aggregation, of two formulation of Clopidogrel (Plavix manufactured by Aventis France vs Pidogrel manufactured by Medis Tunisia). The study was balanced, randomized, two-treatment, two-period, single dose, crossover, in 36 (17 females and 19 males) healthy, adult, human subjects, with a wash out period of 7 days.
The subjects received a single dose of 75 mg following a 12 hour fast but with a standardized breakfast 2 hours following the dose. Clopidogrel blood levels were determined using an HPLC on samples obtained at: Pre-dose and at, 30, 60, 75, 90, 120, 240, 360, 480, 600, 720 and 1440 minutes following drug administration. The bioactivity studies (inhibition of platelet aggregation was performed on the samples collected at 0,120,360,600 and 1440 minutes. The bioactivity was determined using two methods: reduction of surface coverage (using the impact-R instrument from DiaMed Switzerland) and the platelet aggregation time using the Behring coagulation time (BCT) from Dade Behring. The Cmax, Tmax were 8.78 ng/ml, and 2 hours for Pidogrel vs 9.16 ng/ml and 2 hours for Plavix respectively. For both formulations there was an increase (not significant) in platelet aggregation from base line at 2 hours past the dose. Platelets aggregation was inhibited equally by the two products at six hours post dose as measured by the two methods. The platelets of 2 subjects were resistance to the effect of both formulations. Our results indicate that both formulations have comparable bioavailability and bioactivity under the conditions tested. It is recommended to determine the Clopidogrel bioactivity prior to and six hour post the does for all the patients who need the Clopidogrel therapy.

Integrated 'OMIC' Analyses Of The Rat Brain: Novel Biomarker Candidates For Mental Disorders And Stress

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Background: It has been well known that stress may cause mental disorders. However, the process from stress to disorders is not clear yet. Aims: 1) To clarify the effects of several kinds of stress on the brain. 2) To understand mechanisms of animal models for mental disorders, such as depression and developmental disorders. 3) To determine multiple stress markers that may play important roles in mental disorders.
Methods: This study included 280 rats. We prepared animal models for attention-deficit hyperactivity disorder (ADHD) and depression. For ADHD, male rats at 5 days of age received intracisternal injection of 6-hydroxydopamine (6-OHDA) or environmental chemicals. Male congenic wiggling (Wig) rats were generated using Long-Evans and Wistar strains. For depression, adult male rats were exposed to the stress, such as immobility or water. Moreover, we examined the effects of continious light, gamma knife treatment and alcohol drinking. These brains were analyzed by transcriptomics using DNA microarray and proteomics with two-dimensional gel electrophoresis followed by mass spectrometry. In some experiments, we performed metabolomics by NMR.
Results: A deficit in the development of dopamine (DA) neurons caused behavioral hyperactivity similarly to ADHD. Alterations in the expression of genes and proteins in brain regions showed variation among environmental chemicals and differed from those of 6-OHDA-injected and Wig rats. With these techniques, we found stress marker candidates that were similarly altered by immobility, water, and continuous light. Among these, we observed that coffee bean aroma attenuated the effects of water stress (sleep deprivation). Moreover, several stress marker candidates were found after gamma knife treatment and alcohol drinking.
Conclusions: 1) Deficient development of DA neurons may underlie motor hyperactivity, and additional factors may be altered in Wig rats and animals exposed to environmental chemicals, which may reflect different types of ADHD patients. 2) Different kinds of stress that may cause depression altered similar potential biomarkers in the brain. 3) OMIC tools will be useful to study possible alterations in the expression of multiple factors in the brain.

Vitamin K_3 Is A Potent Inhibitor Of Angiogenesis And DNA Polymerase

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Background: Recent studies demonstrate that vitamins not only play essental roles in cellular reactions but also suppress angiogenesis. Angiogenesis is involved in various diseases including cancer, atherosclerosis and diabetic rethinopathy. Thus, anti-angiogenic activity of vitamins may contribute to human health. We have also shown that vitamin B_6 and D_3 and vitamin E derivative, which have anti-angiogenic activity, inhibit DNA polymerase. Althgouh anti-angiogenic activity of vitami K_2 was demonstrated, it remained unknown whether vitamin K_3 had the activities. In this study, we show anti-angiogenic activity of vitami K_3 and the mechanisms and its inhibitory effect on DNA polymerase activity.
Methods: Anti-angiogenic activity of vitamin K_3 was ascertained in an ex vivo angiogenesis model using a rat aortic ring. To elucidate the mechanisms, its effect on in vitro angiogenesis models using human umbilical vein endothelial cells (HUVECs) with regard to HUVEC growth, tube formation and chemotaxis were evaluated. Effect of vitamin K_3 on DNA polymerase activities was also examined.
Results: Vitamin K_3 suppressed angiogenesis in a rat aortic ring assay. HUVEC tube formation and proliferation were suppressed at 25 □M and 5 □M, respectively. These inhibitory effects were dose-dependent maners. HUVEC migration induced by vascular endothelial growth factor (VEGF) was also suppressed by vitamin K_3 at 5 □M. Vitamin K_3 selectively inhibited DNA polymerase □, however vitamin K_1 and K_2 had no effect on DNA polymerase activities.
Conclusions: 1) Vitamin K_3 exerted anti-angiogenic activity through inhibiting important angiogenesis processes. 2) Vitamin K_3 selectively inhibited DNA polymerase □, however vitamin K_2, which has anti-angiogenic activity, had no effect. 3) Vitamin K_3 could be a potent anti-cancer agent.

Global And Local Structure Of Cisplatin And DNA Base Pair Complex: A Theoretical Study

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Background: Since cisplatin was discovered by Rosenberg in 1969, platinum complexes have received much attention for their effect as antitumor drugs. Although other anticancer drugs now replace cisplatin, it is used as a benchmark comparator for new drugs. It causes a cell disorder that leads to apoptosis of the living cell. The distorted DNAs are observed in X-ray analysis at 1.65–2.50 Å resolutions and in NMR experiments. Experimentally, it is known that the bridged structure consists of 65% 1,2-d (GpG), 25% 1,2-d (ApG), and the rest is other bridged structures. There are two possible influences of Pt-DNA formation: 1) global structural changes, such as the distortion of the DNA structure, or 2) local structural changes, such as a DNA mutation. Quantum chemistry enables us to understand the chemical reaction in small molecule systems. The aim of this study is to understand the tendencies of bridged structure qualitatively and to focus on the DNA mutation reactions from the view of computational chemistry. In this abstract, we discuss the base-preference of cisplatin.
Methods: Because of the computational cost, we confined the system extremely small as two base pairs and cisplatin. We used density functional theory (DFT) and ONIOM method. We also assumed that the Pt atom binds to N_7 of G and A. We assumed the binding energy as the energy difference between $[Pt(NH3)4]^{2+}$+ B_1+B_2 and Cis-(B_1-Pt-B_2)+ $2NH_3$.
Results: Following table shows the binding energy of d(B_1pB_2) (in kcal/mol).

Sequence	B_1=G, B_2=G	B_1=A, B_2=G	B_1=A, B_2=A
Energy	73.8	56.1	21.3

The bases are likely to bind to the Pt complex in the order d(GpG), d(ApG) and d(ApA). In particular, the binding energy of the d(ApA) is remarkably low in this calculation.
Conclusions: From the energetics of two base pairs with the cisplatin, it is theoretically confirmed that the Pt complex is likely to bind in the order d(GpG), d(ApG) and d(ApA), and the Pt atom prefers the N_7 site of guanine to that of adenine. This result supports the experimental evidence, where the structure d(ApA) is seldom observed at room temperature. In the presentation, we will discuss the DNA mutation reaction caused by cisplatin binding.

Inhibition Of Tumor Metastasis And Angiogenesis By NK4, Bifunctional Inhibitor Of HGF-Met And Angiogenesis

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Background: Hepatocyte growth factor (HGF) and the Met/HGF receptor tyrosine kinase play a crucial role in invasion and metastasis of a variety of cancer cells. HGF induces dissociation of cell-cell contact, breakdown of extracellular matrix, and concomitant migration of cells, thereby potently enhancing cancer invasion and metastasis. Thus, HGF-Met system is a notable molecular target in cancer therapeutics to inhibit tumor invasion and metastasis.
Results: We prepared NK4, a competitive antagonist for HGF-Met. NK4 is an internal fragment of HGF that encompassing the N-terminal and subsequent four kringle domains. We thereafter found that NK4 acts as an angiogenesis inhibitor as well as HGF-antagonist. NK4 inhibits angiogenesis driven by vascular endothelial cell growth factor and basic fibroblast growth factor, as well as HGF. Through its binding to a protein differrent from Met receptor, NK4 inhibits cell surface assembly of fibronectin and integrin-mediated signaling, thereby inhibiting angiogenesis. Based on unique bifunctional characteristics of NK4, therapeutic approaches with NK4 have been examined. In a model of colon cancer metastasis, NK4 suppressed liver metastasis. In situ Met tyrosine phosphorylation was inhibited by NK4 and this was associated with inhibition of invasion and spreading of metastases in the liver. NK4 suppressed intrahepatic growth of metastases, mainly by inhibiting tumor angiogenesis. In an orthotopic implantation model of pancreatic cancer, NK4 suppressed peritoneal metastasis, tumor growth, and accumulation of ascites, and this was associated with prolonged survival.
Conclusions: The invasive and metastatic behavior of cancer leads to difficulty in attaining a long-term survival. We propose that simultaneous targeting of tumor angiogenesis and the HGF-Met-mediated metastasis by NK4 may prove to be a new approach to treating patients with aggressive tumor.
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The Effects Of Single-Dose Ethanol Administration To Aldehyde Dehydrogenase 2 Knock-Out Mice: Down-Regulation Of Expression Of Cytochrome P450 2E1 Mrna And Amelioration Of Oxidative Stress In Liver Tissue

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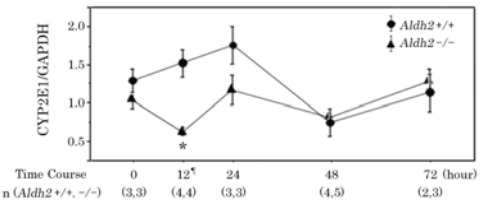
Background: The polymorphism of aldehyde dehydrogenase 2 (*ALDH2*), denoted *ALDH2*2*, is very common in East Asian people. Acetaldehyde, an intermediate metabolite of ethanol, is metabolized very slowly in people with *ALDH2*2* because the mutant ALDH2 protein lacks the activity of acetaldehyde metabolism. On the other hand, one of the cytochrome P450 enzymes, CYP2E1, is well known as an activator of carcinogens and a generator of oxidative stress, and CYP2E1 is induced by ethanol via gene transcriptional regulation. Oxidative stress is also generated when ethanol is metabolized with enzymes other than CYP2E1.
Methods: To examine the consequences of the *ALDH2*2*polymorphism, *Aldh2*+/+ and -/- mice were orally administered ethanol at a dose of 5 g/kg body weight and the levels in liver tissue of CYP2E1 mRNA, malondialdehyde (MDA, an indicator of oxidative stress), and glutathione (GSH, a key antioxidant), were then analyzed 0 - 72 hours after administration.
Results: The level of CYP2E1 mRNA 12 hours after ethanol administration in *Aldh2*-/- mice was significantly lower than that in the 0 hour *Aldh2*-/- group and that in the 12 hour *Aldh2*+/+ group (*, ¶: $p < 0.05$). The levels of MDA were significantly lower in *Aldh2*-/- mice than in *Aldh2*+/+ mice at 12 hours after ethanol administration, while levels of GSH were significantly higher in *Aldh2*-/- mice than in *Aldh2*+/+ mice at 6 and 12 hours after administration.



Conclusions: 1) Single-dose ethanol administration down-regulates the expression of cytochrome p450 2E1 mRNA in the presence of inactive ALDH2. 2) A lack of ALDH2 ameliorates ethanol-induced oxidative stress in liver tissue.

Consideration Of Metachromatic Spectral Changes Of Toluidine Blue Staining Of DNA Depending On Its Helical Structure

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Background: Metachromasy (metachromasia) is a well-known observation in biological and medical experiments. This phenomenon often allows to discriminate a certain substrate or tissue from others and thus metachromatic staining has been used as a cytological and diagnostic tool. Metachromatic colour changes have been considered as being caused by dye aggregation. However, the structure of the dye aggregates is still unknown. In this study, as a first step for our goal to understand structure-function relationships of biomolecules by means of metachromatic staining, the metachromatic spectral changes of Toluidine blue(TB) with DNAs were considered by computational approach.
Methods: TB-DNA complex models were constructed by the optimized molecular structure of TB calculated by quantum calculations (Software: CAChe ver 5.2, Hamiltonian: AM1 for structure optimization and ZINDO/S for electronic properties) and crystal structures of DNAs obtained from Protein Data Bank (B-DNA: 1BNA and A-DNA: 440D). The dimethyl amino group of TB was assumed to bind to a phosphate group of DNAs by electrostatic interaction. Displacement energies for the TB aggregates were estimated by the extended-dipole model using the calculated transition dipole moment of TB corresponding to its visible absorption.
Results: In TB-B-DNA complexation, the two adjacent dye molecules gave a hypsochromic spectral shift in the range from 117 to 330 cm^{-1}. The resulting spectral shifts are strongly related with the geometrical parameters of DNA. The twist angle and the alpha-angle were found to play an important role in the determination of the dimer geometry. A small difference between the alpha-angles of adjacent phosphate groups resulted in a parallel arrangement of TB molecules producing a large spectral shift. In TB-A-DNA complexation, on the other hand, the geometrical limitation for TB-dimer formation was revealed due to a short distance between adjacent phosphate sites. These results suggested that the dimer formation is considerably affected by the helical structure of DNA.
Conclusions: 1) In the complexation of TB with the B-DNA, hypsochromic TB dimers were found to form with the DNA. Their spectral shifts are strongly influenced by the twist angle and the alpha-angle. 2) The dimer formation is sterically restricted in the tightly rolled A-DNA.

Innovations In Organoboron Chemistry Essential To The Discovery Of Bortezomib

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Bortezomib, currently approved for treating relapsed multiple myeloma and undergoing testing for other kinds of anticancer activity, is the first successful proteasome inhibitor and the first useful pharmaceutical containing boron. Before bortezomib could be synthesized and tested, a series of fundamental discoveries in the chemistry of boronic esters was required.
These discoveries began in my laboratory when it was found that halogen atoms on a carbon attached to boron undergo unusually facile boron assisted displacement by nucleophiles (1963). We reported a dialkylamino boronic acid in 1968. Biochemist Gustav E. Lienhard suggested that the boronic acid analogue of N-acetylphenylalanine, $PhCH_2CH(NHAc)B(OH)_2$, ought to be a good chymotrypsin inhibitor (1971). After a variety of failed attempted syntheses, we found that amino boronic esters having an NH group decomposed readily (1979).
Finally, it was found that the facilitation of nucleophilic halide substitution by adjacent boron is so great that lithiohexamethyldisilazane, $LiN(SiMe_3)_2$, could be used to introduce the amino nitrogen, and prompt acylation after protonolysis of the silyl groups led to the stable targeted N-acetylphenylalanine analogue, which was indeed a good inhibitor of chymotrypsin (with K. M. Sadhu and G. E. Lienhard, 1981). In the meantime, other discoveries in my laboratory had led to an efficient general asymmetric synthesis of the requisite chloro boronic ester precursors (1980).
Soon after our initial report, du Pont chemists extended and refined our chemistry to produce several interesting serine protease inhibitors, including highly active elastase and thrombin inhibitors, and studied their mechanisms of action in great detail, but did not find clinically useful drugs. Julian Adams and coworkers reported the activity of bortezomib in 1998 after screening numerous potential inhibitors of proteasome 26S.
There are still unsolved problems regarding the synthesis of highly functionalized organoboron compounds. Among the more interesting of these are boron analogs of nucleosides. Some of the author's current interests and recent progress on these problems will be described.

IM28 Inhibiting HIV1 Replication, Glucose, Lipids, Hemoglobin Levels And Nitric Oxide (NO) In HIV1 Patients: Study From Gabon

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Background: IM28, an analog of dehydroepiandrosterone (DHEA) demonstrates deep in-vitro HIV-1 antiviral activity including, the inhibition of the reverse transcriptase activity, the restriction of envelop proteins mediating cell-cell fusion (gp120/gp41) between infected and healthy cells and the suppression of 3TC/AZT resistant clinical isolate HIV-1. Aim: To study the effects of IM28 or DHEA on a short term pre-clinical trial of six months on 201 HIV-1 patients among which 90 carried opportunistic pathologies such as diabetes, hypertension, tuberculosis, malaria, skin rash, digestive rash and urinal rash, facial paralysis, language troubles, memory deficit, anorexia and anxiety.
Methods: Patients received 50 mg/day/70Kg of IM28 or DHEA and were weekly monitored by our physicians according to the research guidelines of our institution.
Results: No side effects attributable to IM28 were noticed regarding hepatic, cardiac and renal functions evaluation as no significant difference was seen with baseline of urea, creatinine, GOT and TGP. By contrast to patients treated with DHEA, normalization of glycaemia, increased body weight, CD4 ($p<0.01$), lymphocytes and haemoglobin levels ($p<0.001$) paralleled by significant reduction of platelets, antigenemia p24 ($p<0.001$) and viral load ($p<0.01$) were observed in patients under IM28 treatment. In addition, IM28 normalised body weight, lipids, glucose levels and blood pressure in obese, hypertensive and diabetes patients more to reduce significantly the percentage of opportunistic affections such as tuberculosis, malaria, skin rash, digestive rash, urinal rash, stroke, facial paralysis, language and memory troubles, dementia and anxiety. Moreover, the body temperature which is always higher in HIV1 patients and persisting under HAART treatment was reduced and normalised in patients under IM28.
Conclusion: Data suggest that, the use or the substitution of DHEA which is already used as supplement of HIV-1 treatment by IM28 should represent a better therapeutic avenue for HIV-1 and opportunistic related diseases as well as for cardiovascular diseases. Magic bullet effects of IM28 may be partly due by nitric oxide (NO) through the normalisation of his reservoir haemoglobin levels.

A Concerted Approach Using Physical Chemical Methodologies, Computational Chemistry And 3D QSAR Studies Aiming To Develop Novel Analgesic Cannabinoid Analogs

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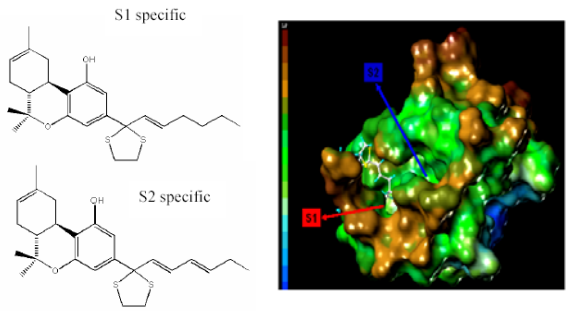
The C-1'-dithiolane Δ^8-tetrahydrocannabinol (Δ^8-THC) amphiphilic analogue (–)-2-(6a,7,10,10atetrahydro-6,6,9-trimethylhydroxy-6H-dibenzo[b,d]pyranyl)-2-hexyl-1,3dithiolane (AMG3) is considered as one of the most potent synthetic analgesic cannabinoid (CB) ligands. Its structure is characterized by a rigid tricycle and flexible alkyl chain segments. Its conformational and ADME properties have been explored using a combination of physical chemical methodologies, computational chemistry and 3D QSAR studies. More particularly, a strategy is developed in which its conformational properties are studied in an ascending complexity. Thus, the conformational properties of AMG 3 are explored in vacuum, in amphoteric solvents, in the receptor site and in a bilayer environment that closely simulates the biological one. Two sites are discovered in the binding pocket (see Figure below) which show distinct conformational preferences and structural requirements (S1 specific and S2 specific). These sites can be explored for a future design and may constitute a magic bullet target for potential analgesic drugs. Two examples of potential magic bullet targets are given below.



Amifostine (WR-2721) As A Cytoprotective Agent

MAZUR L

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Background: Chemotherapy and radiation therapy are the basic approaches in cancer treatment. The current therapies are associated with significant cytotoxicity, as chemo- and radio-therapy do not discriminate between normal and pathological cells. The attempt to increase the therapeutic ratio in an affort to improve survival or quality of life is the goal of modern cancer therapy. The use of cytoprotective agents is one approach to minimizing the toxicity caused by the therapy to normal cells. Aims: 1) to review the vast literature published on amifostine, the aminothiol formerly known as WR-2721, 2) to demonstrate the results of the preclinical experiments and clinical experience with amifostine, 3) to summarize the knowledge on chemo- and radio-protective effects of WR-2721 on normal cells, 4) to show the effects of the aminothiol on programmed cell death, 5) to determine the mechanisms of cytoprotection induced by amifostine, and 6) to provide the insight into future experimental and clinical directions.
Methods: A review of the data bases on a collection of the original papers published and the abstracts of relevant articles searched in the SCOPUS and MEDLINE dabatase.
Results: Amifostine was developed to selectively protect various normal tissues against the hemato-, nephro-, neuro-, and cardio-toxicity, mucositis and xerostomia caused by ionizing radiation and/or chemotherapeutic drugs, e.g. platinum agents, oxazaphosphorines, anthracyclines, taxanes. Amifostine provides a broad-spectrum of cytoprotection against the radio- and chemo-toxicity observed in patients with breast, bladder, cervix, head and neck, non-small cell lung, ovarian, and rectal cancers, melanoma and lymphomas, without attenuating anti-cancer response. Amifostine has potential applications in many oncologic settings, but the precise mechanisms whereby WR-2721 exerts the cytoprotective action on normal cells are not entirely clear. A better understanding of the mechanisms responsible for the cytoprotective effects of Amifostine can considerably broaden its clinical application.
Conclusions: 1) After several decades of preclinical and clinical research, amifostine is widely used in clinical practice as the best known cytoprotector against the adverse effects of chemo- and radio-therapy, 2) As a chemo- and radio-protector of normal cells, amifostine is accepted to be a powerful adjuvant to the current therapies.

The Use of DNA-based Therapies to Restore Clinical Efficacy of Antibiotics

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Background: We are developing DNA-based therapies (transcription factor decoys; TFDs) to treat infectious diseases. TFDs prevent expression of targeted genes within pathogenic bacteria by interfering with the interaction of transcription factors with transcriptional promoters and so preventing expression. We have identified TFDs capable of preventing induction of antibiotic resistance genes and used them to mediate antibiotic resistance in pathogenic bacteria. This technology has the potential to tackle the phenomenon of multiple-drug resistant (MDR) hospital infections- a problem for which there is no ready solution.
Methods: TFDs are designed rationally by examination of sequence literature and data mining of microarray studies where regulators of virulence and antibiotic resistance have been determined in pathogenic bacteria. TFDs incorporate such binding sites and are derivatized with lipids and peptides to aid transfection into bacteria. There effect on growth and viability is monitored in 96 well plate assays and uptake and stability of the TFD measured by microscopy and quantitative PCR.
Results: TFDs have been transfected into examples of both Gram-negative and –positive bacteria to control genetic induction and modify patterns of cell growth, particularly in response to antibiotic stress.
Conclusions: TFDs have the advantages that they mobilize genetic information directly into the clinics (decreasing development timeline) and the generic logic of the approach means it can be applied to many targets. Hence, it has the potential to supply a new class of therapeutics that can be quickly designed, cheaply produced and capable of preventing induction of many drug resistance phenotypes. As TFDs work by targeting DNA-protein interactions controlling an essential genetic switch it becomes more difficult for the pathogen to acquire resistance as either both the genomic binding site and its cognate transcription factor would have to change or the pathogen would need require a genetically distinct antibiotic resistance pathway.

The Hyperglycaemic Effect Of S-Nitrosoglutathione And S-Nitroso-N-Acetylpenincillamine – Possible Mechanism Of Action

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Background: Nitric oxide (NO) is an important bioactive signaling molecule that mediates a variety of normal physiological functions, which, if altered, could contribute to the genesis of many pathological conditions, including diabetes. The study investigated the effects of NO released from S-nitroso-N-acetylpenicillamine (SNAP) and S-nitrosoglutathione on blood glucose levels. Any possible dibetogenic effects of SNAP and GSNO was done by examining the binding of insulin to its receptor on the cell membranes of mononuclear leucocytes from dogs. In addition, the role of NO released from GSNO and SNAP on glucose uptake in skeletal muscle of normoglyceamic and type 2 diabetic rats were examined.
Results: The oral glucose tolerance test revealed an impaired glucose tolerance in GSNO- and SNAP-treated dogs. Mononuclear leucocytes from SNAP-treated dogs had decreased ability to bind insulin (16.30 +/- 1.24%) when compared with those from captopril-treated controls (20.30 +/- 1.93%). Scatchard analysis demonstrated that this decrease in insulin binding was accounted for by a decrease in insulin receptor sites per cell, with mononuclear leucocytes of SNAP-treated dogs having 55 % less insulin receptor sites per cell compared with those of captopril-treated controls ($P < 0.05$). The abnormality in glucose metabolism on administration of GSNO was attributed to decreased binding of insulin to its receptor on the cell membrane of mononuclear leucocytes, 11.60 +/- 0.60% in GSNO-treated dogs compared with 18.10 +/- 1.90% in captopril-treated control ($p < 0.05$). The decreased insulin binding was attributed to decreased insulin receptor sites per cell, 21.43 +/- 2.51 x 10^4 in GSNO-treated dogs compared with 26.60 +/- 1.57 x 10^4 in captopril-treated controls ($p < 0.05$). In rats, 10 mM & 20 mM of GSNO and SNAP significantly decreased basal and insulin-stimulated glucose uptake in skeletal muscle strips of normoglycaemic and type 2 diabetic rats in a concentration-dependent manner ($P<0.05$). The inhibition of insulin-stimulated glucose uptake was greater in the diabetic rats using both NO donors compared with normoglycaemic rats ($P<0.05$).
Conclusion: These findings suggest the first evidence of the novel role of NO as a modulator of insulin binding and glucose uptake, and the involvement of NO in the etiology of diabetes mellitus.

Paul Ehrlich And The Search For Magic Bullets

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The College of Pharmacy offers an Honors Program course to attract students to pursue careers as professional practitioners and/or as pharmaceutical/medical scientists. The course is designed to focus on the continuing search for magic bullets (MBs) as first described by Paul Ehrlich.
The Honors Seminar is a three-credit semester course that incorporates historical, social, and scientific perspectives about the development of medications that attempt to meet the ideal characteristics of a MB. A historical review of Paul Ehrlich's life is presented with an emphasis on his research and development of a MB for the treatment of syphilis. Ehrlich's concept of the MB is the context for presentations on pharmaceutical research. Students are consistently asked to evaluate whether a new medication is truly a MB as envisioned by Ehrlich.
The objectives of the course are: describe the benefits of drug research; discuss the concept of pharmaceutical care; explain the concept of a MB in terms of definition and limitations; state the FDA approval process; state adverse consequences of inappropriate drug use; describe the scientific process for drug discovery; state selected mechanisms of action of drugs; describe research projects by pharmacy faculty.
The topics include: Ehrlich and the Magic Bullet: Ethical Considerations in Drug Research, Bringing a MB to Market; Are Herbal Products MBs?; Historical Perspectives on Drug Tragedies; Pharmaceutical Care: Aiming a MB; Animal Research to Develop MB; MBs to Treat Cancer; MBs to Prevent and Dissolve Clots; MBs for Migraine Headaches; MBs for Alzheimer's Disease; Growth Hormone as a MB; MBs with Food. Does it Matter?; MBs for Asthma; Antibiotics as MBs; MBs for HIV/AIDS; MBs for Acute Otitis Media; MBs for Epilepsy; Are Vaccines the Ultimate MB?; A Debate: Is Marijuana a MB?; MBs for Obesity?; Pharmacogenomics and Drug Response.
Both pharmaceutical and clinical scientists constitute the faculty. This combination of faculty expertise mirrors the scientist-clinician role of Ehrlich, which imparts significant perspectives about research and the use of medications.
Many of the students who have taken this course have continued their education in pharmacy or medicine or pursued graduate studies in the pharmaceutical and medical sciences. Perhaps one or more will emulate Paul Ehrlich's contributions to science and healthcare.

Effect Of Commonly Prescribed Nsaids, Proton Pump Inhibitor And Newer Anti-Malarial Compound On Pharmacokinetics Of Different Antiepileptics

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Background: The aim of the study was to determine the effect of newer NSAIDs, Proton Pump Inhibitor, artemisinin compound on the pharmacokinetics of phenytoin and carbamazepine in rabbits.
Methods: In a parallel design study, phenytoin (30 mg/kg/day) and carbamazepine (40 mg/kg/day orally) were given daily for 7 days in 12 rabbits. On day 7 and 14th day, blood samples were taken at various time intervals between 0 and 24 h. Animals were treated with etoricoxib, acceclofenac, esomeprazole, rabeprazole, artemisinin, artemether, and arteether from 7 day onwards to 14th day. Plasma phenytoin and carbamazepine levels were assayed by HPLC, and pharmacokinetic parameters were calculated.
Results: Treatment with etoricoxib and aceclofenac, there was a decrease in t(1/2)a and t(1/2)el significantly as compared to phenytoin and carbamazepine group alone. Significant changes were observed in the pharmacokinetic parameters in etoricoxib and acceclofenac treated group. With esomeprazole and rabeprazole, there was a decrease in the AUC_{0-24} when carbamazepine and phenytoin was co-administered with esomeprazole. The decrease in AUC_{0-24} (22.78±4.71 to 10.46±2.29), C_{max} (2.76± 0.77 to 1.412±1.08), T_{max} (2.83±0.17 to 3±0.40) was statistically significant ($p<0.05$). In the artemether group, t(1/2) el decreased compared to that of controls. In the arteether group, no significant change was observed in the pharmacokinetic parameters, when carbamazepine was co-administered with artemisinin, artemether or arteether. The increase in AUC(0-infinity) (22.78 +/- 4.71 to 63.10 +/- 12.29), Cmax (2.76 +/- 0.77 to 7.02 +/- 1.08), Tmax (2.83 +/- 0.17 to 4.16 +/- 0.40) was statistically significant when artemether was given along with carbamazepine ($p < 0.05$).
Conclusion(s): These results suggest that newer NSAIDs, Proton Pump Inhibitor, and artemisinin compounds alter the pharmacokinetics of phenytoin and carbamazepine. Confirmation of these results in human studies will warrant changes in phenytoin and carbamazepine dose or frequency, when either of these drug is co-administered with it.

Direct Lentiviral Injection Induces Potent Anti-CEA Immunity In CEA Transgenic Mice

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Background: Immunotherapy would be dramatically broadened to more recipients if direct 'off-the-shelf' products could be engineered to engender functionally potent immune responses against true 'self' tumor antigens. The carcinoembryonic antigen (CEA) is upregulated in colon cancers, non-small cell lung cancers, and breast cancers. CEA is an excellent and important target for cancer immunotherapy.
Methods: The human CEA cDNA was used to construct the lentiviral vector (LV-huCEA). huCEA Tg mice were used for anti-tumor immunity studies. A murine gastric carcinoma cell line expressing huCEA was used to establish subcutaneous tumors. Mice were grafted at day 0 with 0.8×10^6 mGC4CEA tumor cells in the flank and subsequently immunized in the footpad on days 14 and 21 with PBS or 0.15×10^6 transducing units (TU) of LV-enGFP or LV-huCEA. Antibody was measured and splenocytes were used unstimulated in cytokine analyses. Tetramer staining was performed against the EAQNTTYL immunogenic peptide. Tumor sections were stained for immunofluorescence.
Results: We show stabilization (mostly reductions) of 14-day established subcutaneous mGC4CEA tumors in human CEA-transgenic mice following two direct low-dose injections of LV-huCEA and not LV-enGFP. This stabilization result was reproducible and detailed analyses including antibody assays, multiplex cytokine analyses on unstimulated splenocytes, tetramer staining, and immunofluorescence staining of tumor sections demonstrated that this outcome correlated with both a cellular and humoral immune response.
Conclusions: We observed that we can safely break tolerance to huCEA and engineer an efficient anti-tumor response along with anti-huCEA antibody and CTL responses. These data support the use of direct injections of low doses of LV-huCEA for enhancement of tumor immunotherapy directed against CEA.

Bromocriptine Effect In Cardiorenal Damage In Patients With Type 2 Diabetes Mellitus

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Background: Left ventricular hypertrophy (LVH) predicts cardiovascular morbidity in patients with diabetic nephropathy and chronic kidney disease (CKD). Its prevalence increases as the renal function declines. Bromocriptine a DA2 receptor agonist inhibits norepinephrine release, aldosterone secretion, the expression of type-1 angiotensin II receptors. The aim of this study was to analyze the effect of BEC on LVH and its influence in residual renal function in patients with LVH, diabetic nephropathy and stage IV of CKD.
Methods: A 6-month double blind randomized controlled clinical trial was conducted in 28 diabetic patients. Fourteen patients received bromocriptine (BEC) (2.5 mg tablet trice a day), 14 received placebo (PLO) (1 tablet trice a day), Changes in left ventricular hypertrophy (assessed by two dimensionally guided M-mode echocardiography). Blood pressure (24h ambulatory blood pressure monitoring), 24h creatinine clearance, cystatin pro brain natriuretic peptide (pBNP), prolactin and other biochemical determinations were assessed at baseline, three and six months of follow-up. The repeated measures analysis was done.
Results: Bromocriptine significantly reduced from baseline: left ventricular mass (-46±8.35g), left ventricular mass index (-28.28 g/m^2), interventricular septum (-1.14 mm), left ventricular diastolic diameter (-1.8 mm) and left ventricular posterior wall thickness (-1.71±0.46); BNP (-1.51±1.1 pg/ml) $p < .001$. Mean blood pressure (-4.23±0.68 mmHg). 24h creatinine clearance and cystatin levels didn't change in the BEC group. No differences from baseline were observed in echocardiography parameters in the PLO group. However, these patients had reduction in 24h creatinine clearance and increased cystatin levels. Normalization of cholesterol and glucose levels was reached earlier in patients receiving BEC than those in the PLO group.
Conclusions: 1. - BEC treatment reduced: left ventricular mass, blood pressure, pBNP and plasma prolactin levels. 2. - It prevented the decline of residual renal function. 3.-It improved the metabolic control.

Are The Substituted Phenethylamines Magic Bullets? Benefits And Harms Of Amphetamines And Related Compounds

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The substituted phenethylamines (including amphetamine, methamphetamine and methylenedioxymethamphetamine; MDMA) are potent therapeutics and major drugs of abuse. Amphetamine was first synthesized in 1897 in Berlin and is one of medicines oldest synthetic therapeutics. Substituted phenethylamines have demonstrated efficacy in treating conditions as diverse as nasal congestion and attention deficit disorder. When used for defined indications and under medical supervision substituted phenethylamine compounds are among the safest and most effective medications ever developed – magic bullets by Ehrlich's definition. However, magic bullets can harm if not used appropriately. Over the last 50 years the substituted phenethylamines methamphetamine and MDMA have become major drugs of abuse; methamphetamine is the most widely abused synthetic drug in the world with high rates of addiction in all developed countries. In this presentation the pharmacology of the substituted phenethylamines will be reviewed. Therapeutic indications, non-medical military and athletic uses, abuse and addiction will be discussed and pharmacologic mechanisms described. We have conducted several studies defining the human pharmacology of MDMA and methamphetamine. Our work suggests that the pro-social effects of MDMA are mediated by increases in oxytocin release and MDMA alter recognition of emotional stimuli. Phenethylamine has a chiral center and there are substantial differences in the pharmacologic effects of the substituted phenethylamine stereoisomers – data describing these differences will be presented. Due to widespread abuse of phenethylamine compounds there has been little interest in developing new phenethylamine therapeutics. In this presentation we will advance the hypothesis that many undiscovered magic bullets exist within this class of compounds and that more investigation and interest is warranted.
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Antiviral Effect Of Lamivudine, Emtricitabine, Adefovir Dipivoxil, And Tenofovir Disoproxil Fumarate, Administered Orally Alone And In Combination, To Woodchucks With Chronic Woodchuck Hepatitis Virus Infection

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Adefovir dipivoxil (ADV) and tenofovir disoproxil fumarate (TDF) are nucleotide analogs that inhibit replication of wild-type hepatitis B virus (HBV) and lamivudine-(3TC) resistant HBV in patients, including patients co-infected with the human immunodeficiency virus (HIV). Combination of ADV or TDF with other nucleoside analogs is a proposed strategy for managing antiviral drug resistance during treatment of chronic HBV infection. The antiviral effect of oral administration of ADV (15 mg/kg/day) or TDF (15 mg/kg/day), alone or in combination with 3TC (15 mg/kg/day) or emtricitabine (FTC; 15 mg/kg/day) against chronic woodchuck hepatitis virus (WHV) infection was evaluated in groups of 5 woodchucks for 48 weeks. Dosages were chosen due to their efficacy in previous treatment studies in woodchucks. Once-daily treatment with the combination of ADV+3TC (4 survivors) or of TDF+FTC (4 survivors) significantly reduced serum WHV viremia from pretreatment level by 6.2 and 6.1 $\log_{10}$, respectively, as determined by dot-blot hybridization and real-time PCR (P<0.01). Additional findings include treatment with TDF+3TC (4 survivors, 5.6 $\log_{10}$), ADV (4 survivors, 4.8 $\log_{10}$), ADV+FTC (1 survivor, 4.4 $\log_{10}$), TDF (3 survivors, 2.9 $\log_{10}$), 3TC (5 survivors, 2.7 $\log_{10}$), and FTC (5 survivors, 2.0 $\log_{10}$). Individual woodchucks across all treatment groups also demonstrated pronounced declines in serum WHV surface antigen as determined by ELISA. Characteristically, these woodchucks also had declines in hepatic WHV replication and hepatic expression of WHV antigens as determined by Southern and northern blot hybridization or immunohistochemistry, respectively. Most woodchucks had prompt recrudescence of WHV replication following drug withdrawal, but individual woodchucks across treatment groups had sustained effects. No signs of toxicity were observed for any of the drugs and drug combinations administered. In conclusion, oral administration of 3TC, FTC, ADV, and TDF, alone and in combination was safe and effective in the woodchuck model of chronic HBV infection.

Arsenic Trioxide Induces Apoptosis Preferentially In B-CLL Cells Of Patients With Unfavorable Prognostic Factors Including Del17p13

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In the last decade, arsenic trioxide has been used very successfully to treat acute promyelocytic leukemia (APL). Much less is known about the effectiveness of arsenic trioxide in other neoplastic disorders. We report here that after 18 h in vitro treatment with 4 µM arsenic trioxide, 75 ± 18 % of B-CLL cells (n = 52) underwent apoptosis. Importantly, B-CLL cells harboring a deletion of chromosome 17p13, which predisposes to fludarabine resistance and has been identified as an important negative predictor of clinical outcome, were more susceptible to arsenic trioxide toxicity than cells lacking this aberration. Furthermore, unfavorable risk profiles such as unmutated IgVH status, high CD38 expression and prior treatment were associated with significantly higher sensitivity of B-CLL cells to arsenic trioxide. Arsenic trioxide also preferentially killed B-CLL cells as compared to B-cells from healthy age-matched controls. Molecular analysis revealed that basal superoxide dismutase activity was positively correlated with the pro-apoptotic activity of arsenic trioxide pointing to a role of reactive oxygen species in cell death induction. The high activity of arsenic trioxide in B-CLL cells from high-risk patients makes it a promising drug for high-risk and/or fludarabine-refractory B-CLL patients.

Gene Expression In Brain And Kidney In Response To Aluminum In Drugs

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Background: Aluminum (Al) has been used in various drugs such as analgesics, antacids and vaccines as well as the domestic uses. It has been believed that the absorption rate of Al from the intestine is quite low and the absorbed Al is excreted rapidly into urine. However, it is not known whether Al remains in the body in the case of the intake of Al-containing drugs. Al has been linked to such diseases as dialysis encephalopathy, osteomalacia, □2-microglobulin-associated amyloidosis, amyotrophic lateral sclerosis and parkinsonisms-dementia in the Kii Peninsula and Guam. Recently the mechanism of adjuvant of Al in immune system has been reported. Therefore, the influence of Al on gene expression in relation to diseases has been interested in.
Methods: Al in human urine was analyzed by graphite furnace atomic absorption spectrometry with a Zeeman background correction. The gene-expression in mice was widely screened by differential display analysis. The corresponding genes were identified by sequencing. Gene expression was confirmed by RT-PCR and the corresponding proteins were identified by Western blotting in cultured cells and animals.
Results: The absorption rate of Al from the intestine was very low, but it was shown that the consecutive intake of Al-containing drugs exhibited extremely high excretion of Al in urine for days through the examination of volunteers. As a result it was confirmed that Al remained for a long period and circulated in the body. Al did not enter the brain in a normal condition, but *in vitro* astrocytes revealed apoptotic cell death, in which the gene expression and protein production in mitochondria did not change but those of endoplasmic reticulum altered. It was found that Al caused up-regulation of several gene-expression in kidneys. In further experiments it was confirmed to increase the renin production in response to relatively low concentration of Al.
Conclusions: Al remains for a long period and circulates in the body. Even at a very low concentration of Al the cultured cells revealed the ER stress as the alteration in gene expression. Particularly, it is pointed out that a chronic exposure of Al may be a cause of essential hypertension due to the up-regulation of renin.

Psychoncology And Psychoneuroendocrinoimmunology (PNEI): Relation Between The Anticancer Immunity And The Psychospiritual Status Of Cancer Patients

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It is known that the psychospiritual status may influence tumour growth and the prognosis of the cancer. Only with the development of PNEI it has been possible to characterize the neuroimmunochemical mechanism responsible for the influence of the psychospiritual condition on tumour growth, through the modulation of the immune system. The anticancer immunity is stimulate by IL-2 and IL-12 whereas it is inhibited by IL-10 and IL-6. It has been observed that the evidence of low of IL-6 an IL-10, low number of lymphocytes and T-helper lymphocytes and an increase number of T reg is associated with the poor prognosis. Within the great number of the psychological variable, the spirituality has to be considered as different from the psychological profile. According to the PNEI discoveries we have performed several studies to investigate the psychological profile of cancer patients in relation to their psychoneuroendocrinoimmune status. The psychological profile was investigate by the Rorschach's test and the spiritual status was analyzed by special spiritual test. We are performed five main studies, by obtaining the following results: 1)low number of lymphocytes and T-helper lymphocytes in patients with suppression of spiritual and sexual sensitivity; 2)low efficacy of IL-2 immunotherapy and low lymphocytes response to IL-2 in patients with anxiety and or loss of sexual identity; 3)low number of lymphocytes associated with alteration in the circadian rhythm of cortisol and hypercortisolemia in patients with low spiritual profile; 4)abnormally high percent of T -regulatory lymphocytes in patients showing self-punishment status; 5)lack of surgery induced-hyperprolactinemia in operable breast cancer patients with suppression of the maternal behaviour. This results would suggest the possibility to investigate the psychoneuroimmune basis of the overall psychological profile in cancer patients. Further studies by investigating the brain endocannabinoid system through the detection of the blood concentration of the main endocannabinoid agent anandamide will clarify the neurochemical alteration responsible for the progressive loss of the pleasure in neoplastic disease.

Anticancer Gold Compounds: Mechanistic Insights

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Background: Gold compounds are a class of metallodrugs, of potential great interest for cancer treatment. During the past two decades a large variety of gold(I) and gold(III) compounds were shown to manifest relevant antiproliferative properties in vitro against selected human tumor cell lines qualifying themselves as excellent candidates for further pharmacological testing. On turn, mechanistic studies pointed out that the interactions of cytotoxic gold compounds with DNA are generally far weaker than those of platinum drugs implying a different mode of action. The main goal of our investigation is to provide new insight into the possible mechanisms of action of this variegate family of cytotoxic drugs.
Methods: A variety of methods were employed to disclose the mode of action of gold drugs. Several gold compounds were prepared according to classical methods of coordination chemistry and extensively characterized in the solid state and in solution. Their biological properties were evaluated both at the molecular and the cellular level. Their effecs on selected protein targets were investigated. In vitro cytotoxic properties toward a wide panel of human tumor cell lines were measured.
Results: A certain number of gold compounds, in most cases in the oxidation state +3, were prepared and characterised that manifested very pronounced cytotoxic effects in vitro. For most of them potent inhibition of thioredoxin reductase was established. It is proposed that thioredoxin reductase inhibition triggers cell apoptosis through a mitochondrial pathway. Morevover, extensive data have been collected on the cellular effects in vitro of a panel of gold compounds and specific insight into their respective mechanisms of action has been achieved.
Conclusions: Gold(III) compounds constitute a novel family of cytotoxic drugs of great interest as potential anticancer agents. Their cytotoxic effects are mediated by a variety of molecular mechanisms that are deeply different from those of platinum compounds.

The Knowledge And Expectations Of Parents About The Role Of Antibiotic Treatment In Upper Respiratory Tract Infection (URTI) – A Survey Among Parents Attending Tertiary Care Institution With Their Sick Child

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Introduction: Parents' knowledge, attitudes and expectations of antibiotics have been identified as causes for antibiotic misuse in paediatric practice.
Methods: Parents accompanying their sick children with an URTI, attending at the out patients department and Professorial Paediatric unit of Colombo North Teaching Hospital, Ragama were interviewed by four qualified doctors for their knowledge, attitudes and practices during similar illness. The knowledge of antibiotics was assessed using pictures and samples of antibiotics.
Results: 235 parents (230 females) mean age 31.9 years (SD: 7.33) participated in the study. The level of education in the population was; below grade 5: 11 (4.7%), Grade 5-10: 142 (60.4%), grade 10-12: 71 (30.2%) and higher education: 11 (4.7%). Of the 235, 201 (85.1%) identified antibiotics as a component of their treatment. However only 11 (4.7%) knew they were against bacterial infections; 212(90.3%), 189 (80.8%), 176 (75%), 165 (70.4%), 130 (55.4%), 77 (32.8%) and 55 (23.6%) identified them as treatment for either or combinations of cough, fever, phlegm, cold, sore throat, ear ache and headache. While 116 (49.3%) thought that above treatment was important for cure of illness 119 (50.3%) thought them to be important for early recovery. The expectation of an antibiotic for an URTI (always, 75%, 50%, 25% and never) was 28 (12%), 39 (17%), 23 (10%), 119 (51%) and 23 (10%) respectively. Twenty (8.5%) had requested an antibiotic when it had not been prescribed; 12(60%) from the pharmacy and 8 (40%) from the doctor. Of the 235, 172 (73%) claimed to complete the full course of treatment while 18 (7.8%) keep the excess antibiotics for future use.
Conclusions: The knowledge, expectation, demand and self medication of antibiotics seems to be lower among parents in our population compared to European populations.

Bone Marrow Stromal Cells Attenuate Sepsis And Sepsis-Induced Acute Kidney Injury (AKI) Via A Novel Mechanism Of Action

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Background: Acute kidney injury (AKI) in septic shock is associated with a high mortality in ICU patients. Bone marrow stromal cells (BMSCs), which are often referred to as mesenchymal stem cells, have been shown to improve outcome in a number of different animal injury models by altering inflammation, apoptosis, and necrosis. In this study we set out to determine whether BMSCs can attenuate the severity of sepsis-induced AKI.
Methods: Sepsis was induced in C57/BL6 mice using cecal ligation and puncture (CLP). BMSCs were obtained from the bone marrow of 6-8 week old mice.
Results: Intravenous administration of BMSCs (10^6 cells/mouse) immediately before CLP surgery resulted in significantly longer survival and improved kidney, liver and pancreatic function. Such organ protection was not obtained with either hematopoietic stem cells or necrotic BMSCs. Searching for a mechanism of action we found that 24 hs after CLP the serum levels of proinflammatory cytokines (TNF-□, IL-6), peritoneal and kidney vascular permeability, splenic apoptosis and blood bacterial count were significantly reduced in BMSC-treated animals vs controls; the level of IL-10, an anti-inflammatory cytokine, was not affected. Six hours after their injection, fluorescently-labeled BMSCs were detected mostly in the lung. By prelabeling BMSCs with quantum dots and later performing immunostaining with a macrophage marker (Iba-1) we found the BMSCs adjacent to macrophages in the lung. Some were in the spleen, and rare cells were seen in the kidney; 24 hours after they were injected, we found few BMSCs in any organ. The positive effect of BMSC treatment was still present in Rag-/- mice; in NK cell depleted mice, and in IFN-□-/- mice, suggesting that B,T and NK cells, and IFN-□do not play a significant role in mediating the effects of the BMSCs On the other hand, macrophage depletion or pretreatment with IL-10 or IL10 receptor antibodies eliminated the beneficial effects of BMSCs in the sepsis model.
Conclusion: Our results suggest that BMSCs may act on tissue macrophages resulting in enhanced production of IL-10, a potent anti-inflammatory cytokine.

Novel Antineoplastic Agent – Peptide Conjugates As Drug Delivery Systems For Targeted Chemotherapy

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Background: Receptor-mediated tumor-selective drug targeting by peptides might be an efficient tool in cancer therapy. The combination of appropriate drugs with a targeting moiety that recognizes tumor-specific or overexpressed receptors on tumor cells might lead to highly efficient chemotherapeutic agents.
Aims: 1) To develop new conjugates containing antineoplastic agents and peptides with own biological activity. 2) To study the structure-activity relationship *in vitro*. 3) To optimize the *in vivo* treatment of the selected compound.
Methods: Flow cytometry was used with FITC-labeled annexin-V and PI stain, to discriminate intact cells from apoptotic and necrotic cells (MonoMac6) after treatment with the oligotuftsin-Mtx conjugates and Mtx (10^{-8}-10^{-6} M). *In vitro* antitumor activity was studied by MTT assay on MCF-7 human breast and C26 murine colon carcinoma cell lines. Treatments (*i.p.*) of 5-7 mice/group with free Daunorubicine (1x5 mg/kg or 5x2 mg/kg) or Dau-GnRH-III oxime conjugate (1x15 mg/kg or 5x5 mg/kg) were carried out on day 7 followed by repetition every second day in case of lower doses. The conjugate was applied in 2x15 mg/kg on days 4 and 7 or 7 and 10 after tumor transplantation.
Results: 1) Methotrexate-conjugate had cytotoxic effect. 2) Anthracycline-GnRH-III conjugates with different linkages had the following IC_{50} values (□M): ester (0.8) < hydrazone (1.5) < oxime (3.9) < amide (>100) on MCF-7 cells, while somewhat higher on C26 cell line. 2) Dau-GnRH-III oxime conjugate had antitumor activity and it was not toxic up to 15 mg Dau content/kg body weight. The best result was ~50% tumor growth inhibition and 33% increase of survival time to the control on C26 tumor bearing mice in case of treatments on days 4 and 7 after tumor transplantation.
Conclusions: 1) Efficient drug delivery into monocytes and macrophages was obtained by the application of tuftsin-like carriers. 2) The type of ligation had an influence on the antitumor activity. 3) Dau-GnRH-III oxime conjugate prevented the toxic side effect of Dau even at a concentration higher than the lethal dose of the drug. Significant decrease of the tumor growth and increased survival time was determined in case of treatments with Dau-GnRH-III.

Predicting Optimal Endocrine Treatment Of Hormone-Dependent Breast Cancer Through The Right Bullets And Targets

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Functioning of the estrogen receptor (ER) is a prerequisite for outgrowth of hormone-dependent breast cancer. Anti-estrogens such as tamoxifen, inhibit ER functioning by inducing a structural alteration of the ER, which results in a reduced interaction with the transcription factor complex, ultimately leading to cell death.
We found that modification of the ERα by phosphorylation prevents the structural alterations that are the result of binding of anti-estrogens to ERα. This converts the anti-estrogen tamoxifen into an agonist. Each different anti-estrogen demands for its specific modification(s) to render ERα resistant, providing a resistance "signature" (the target) for each anti-estrogen (the bullet).
We generated an antibody that detects one of these modifications; the ERα phosphorylated at Serine 305 by Protein kinase A, and could identify resistance to tamoxifen in a group of breast cancer patients. Modifications in ER by phosphorylation provide a diagnostic tool for personalized breast cancer treatment, since alternative anti-estrogens are at hand for the signature-positive subgroup. They also guide optimal development of novel anti-estrogens.
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Analysis Of Linezolid Chiral And Achiral Impurities By Capillary Electrophoresis

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Rapid growth of pharmaceutical industry as well as the appearance on the market of large number of new medicinal products, combined with growing knowledge about drugs efficiency and safety, cause the necessity of introducing and developing new analytical techniques. Capillary electrophoresis (CE) is a relatively modern analytical technique which enjoyed for the tremendous interest in the last years.
Linezolid is the first available oxazolidinone antibacterial agent, showing strong activity against Gram-positive pathogens, including multidrug-resistant strains. Linezolid contains a centre of asymmetry at C-5 of the oxazolidinone ring imparting chirality in the molecule. Separation of enantiomers is vital in the pharmaceutical chemistry. The stereochemistry of such compounds can affect their biological activities, therefore developing of method for their purification and separation is crucial. The separation of chiral compounds can be achieved in CE by addition of appropriate chiral selector to the background electrolyte (BGE).
The first stage on linezolid study was to elaborate principles of CE enantioseparation of S-linezolid (biologically active form) from its impurity (R-isomer) and to use this method for quantitative analysis of R isomer only. A simple, fast, accurate, precise chiral CE method was elaborated using 27.5 mM heptakis-(2,3-diacetyl-6-sulfato)-ß-cyclodextrin (HDAS-ß-CD) dissolved in 50 mM borate buffer, pH 9.0 and CE in 15°C, normal polarity, uncoated capillary. Effect of HDAS-ß-CD concentration in BGE low pH was also studied.
NMR study and molecular modeling was performed to improve the level of understanding of the chiral recognition process occurring between linezolid and applied HDAS-ß-CD. NMR spectrometry allowed to estimate the 1:1 complex stoichiometry and to determine the binding constants.
Besides, NMR and molecular modeling were applied for investigations of the host-guest complexation of R- or S-linezolid with HDAS-ß-CD.
The next step of the study was to elaborate simplest and reliable method for determination of linezolid and its 14 achiral impurities.
The application of a sweeping preconcentration enabled fast, simple, accurate, and precise separation of linezolid from all its achiral impurities by CE using UV absorption detection method. Satisfactory separation was possible after less than 19 min of electrophoresis.
Capillary electrophoresis proved to be suitable tool for chiral and achiral determination of linezolid in medicinal products.

Recombinant Factor VIIa (rFVIIa) For The Treatment Of Bleeding In Abdominal Surgery And Cardiac Surgery

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Background: Factor VIIa plays a key role in hemostasis.The aim of this study is clinical evaluation of efficacy of rFVIIa in the treatment of bleeding during and after abdominal and cardiac surgery.
Methods: Meta-analyses of case series studies (n==67) on the treatment of bleeding with rFVIIa with regarding reduction or cessation of bleeding and mortlality. We used the questioners of Novo Nordisk to asses the indications and effectiveness of treatment. We compared the amount of blood loss within 12 hours(hrs) before and within 12 hrs after giving rFVIIa, the dynamic of bleeding. In 10 patients the bleeding started after surgery intraoperatively, in 57 patients severy hemorrhage developed in the postoperative period. We also compared full blood count and laboratory coagulation profile parameters before treatment, 2 and 12 hrs after treatment. In cardiac surgery rFVIIa was administered in 5 to 49 minutes after neutralization of heparin with protamine sulfate. The dosage of rFVIIa was 39.23 ±20.70 µ/kg We used students t-test for statistical analysis.
Results: After administration of first median dose (14.45-81.35µg/kg) rFVIIa bleeding stopped in 47 patients. Markedly decreased in 15patients. 5 patients who didn´t benefit from initial rFVIIa administration received additional drug in dose 38.25 µg/kg with good results.
The average blood loss within 12 hrs before treatment was 2510.00 mL ±1642.07 mL and the average blood loss within 12 hrs after treatment was 1057.75±810.67mL The average dynamic of bleeding before treatment was 216.35±138.82 mL/h and 87.90±67.82mL/h after treatment. Transfusion requirements were reduced for PRBC, FFP, plateletes, and crystalloid and/or colloids. Reduction in transfusion requirements was statistically significant (p≤0,05).
Conclusions: The meta-analysis of series cases showed that in a mean of 85% patients rFVIIa achieved at least a reduction of bleeding and reducing the need for hemotransfusions.

Novel Anti-Cancer Peptides That Cause Reversion, Lysis And Necrosis Of Tumor Cells But Have No Effect On Normal Cells

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During the last several years an increasing number of peptide-based therapeutics have been developed and studied for their efficacy in the treatment of cancer. Many of these peptides are directed against molecules that play central roles in cell growth such as ras-p21 protein and p53. In cancer, the genes coding for these two proteins are most frequently subjected to mutations, deletions or inactivation, events that are at the center of the affected cells' malignant behavior. Our interest is in the identification of peptide domains in each of these two proteins that lead to the alteration of each protein's conformation and, consequently, its biological function in response to oncogenic amino acid (AA) substitutions. We have employed computer-based modeling to design peptides from the *ras*-p21 and p53 protein that block proliferation of cancer cells. By first generating and comparing low energy average structures for oncogenic and wild-type proteins using conformational energy calculations we identified peptide domains from the molecules regulatory sites and that were synthesized for the studies described in this presentation. To accomplish trans-membrane transport, the peptides were linked by their carboxyl terminus to a leader sequence, penetratin, from the *antennapedia* protein.
We have synthesized two *ras*-p21 peptide domains, PNC-7 (AA 35-47) and PNC-2 (AA 96-110). The PNC-7 domain of *ras*-p21 is known to participate in the binding of SOS-guanidine nucleotide exchange protein, GAP, PI3K and RBD of raf-p74. The PNC-2 peptide domain participates in the binding of *ras*-p21 to the *jun*-protein and/or the *jun*-N-terminal kinase. We also synthesized two p53 peptide domains, PNC-27 (AA 12-26) and PNC-28 (AA 17-26) which include the AA region that is directly involved in the binding of p53 to hdm2 which prepares p53 for ubiquitination, cytoplasmic delivery and degradation. Each of the four synthetic peptides was linked to penetratin. Similarly, control peptides including scrambled AA sequences of the selected peptide domains were linked to penetratin. We have tested these peptides' efficacy *in vitro* and *in vivo* against rodent and human cancer cells as well as against normal untransformed cells. The results showed that both PNC-7 and PNC-2 induce phenotypic reversion to their untransformed phenotypes of *ras*-p21^{val12}-transformed BMRPA1.TUC3 pancreatic cancer cells and of *ras*-transformed HT1080 human fibrosarcoma cells. Furthermore, both peptides were potently cytotoxic to human MIA-PaCa-2 pancreatic cancer and U-251 astrocytoma cells while they had no effect on the growth of untransformed normal cells. Control peptides showed no effect on tumor cell growth or survival. We then tested the two p53-derived peptides PNC-27 and PNC-28, that contain the p53 hdm2-binding domain, and observed that they kill effectively a variety of cancer cells (cell lines) and primary cancer cells *in vitro*. We have also shown that PNC-28 is cytotoxic to the metastatic pancreatic cancer cell line BMRPA1.TUC3 *in vivo* without adverse side-effects on the host. Most remarkably, neither peptide has any effect on the viability or growth of untransformed normal cells including hematopoietic stem cells. Several control peptides including PNC-29 (pepide from cytochrome p450), scrambled PNC-27 and the penetratin sequence itself had no effect on cancer cells. We further showed that PNC-27 and PNC-28 induce rapid and complete tumor cell necrosis, and not apoptosis, as shown by the early (1-8h) release of LDH by PNC-27- and, respectively, PNC-28-treated cancer cells in the absence of induction of caspase(s) and of DNA laddering. Pursuing the unique specificity of the PNC-27 and PNC-28 effect on tumor versus normal cells, we identified by immunoblotting hdm2/mdm2 antigen in the plasma membrane of human and rodent cancer but not of normal cells. Moreover, confocal immuno-fluorescence microscopy showed green-(FITC)-labeled anti-PNC-27 antibody (Ab) on the plasma membrane tumor cells co-localized with red-(TRITC)-labeled andi-hdm2 Ab. This finding may help explain the selectivity of PNC-peptides for cancer cells. Ultrastructural studies using gold-conjugated hdm2 and PNC-27 Abs confirmed the co-localization of two proteins suggesting the formation of an oligomeric complex of consisting of several hdm2 molecules. Further examination showed that within minutes of drug treatment the disruption of tumor cell plasma membrane and of mitochondria was evident, whereas the nuclear membranes remained intact supporting the notion of cancer cell death by a necrotic-cytotoxic process. Using spinning-disc confocal microscopy with propidium iodide for membrane leakage and nuclear staining and mitochondrion-specific MitoTracker dyes we have been able to record the rapid membrane- and mitochondrial-destructive events that follow the exposure of cancer cells to PNC-27 but not of untransformed normal cells. Taken together these findings let us propose that these novel peptides, PNC-7, PNC-2, PNC -27 and PNC-28, individually and combined, will help arm the oncologist with a new class of a therapeutics that are selectively and potently cytotoxic to cancer cells.

Blockade Of IL-6 Signalling With A Humanized Anti-IL-6 Receptor Antibody, Tocilizumab, For The Treatment Of Rheumatoid Arthritis

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Background: IL-6 is a proinflammatory cytokine and is known to play important roles in the pathogenesis of rheumatoid arthritis (RA). Several clinical studies have demonstrated that blockade of IL-6 signalling by a humanized anti-IL-6 receptor antibody is highly effective in the treatment of patients with active RA who are inadequately responsive to traditional anti-rheumatic drugs. IL-6 has a variety of biological activities that match many of the symptoms observed in RA patients. The precise mechanisms of action of tocilizumab are still not fully understood, however.
Methods: We reviewed possible mechanisms of action of tocilizumab, based on recently published papers.
Results: The data from Phase III clinical studies confirmed that tocilizumab can improve symptoms (including the number of swollen joints, the number of tender joints, fever, fatigue, anaemia and anorexia) in moderate to severe active RA. It has also been reported that tocilizumab prevented the radiographic progression of joint destruction. Tocilizumab has generally been well tolerated. Interestingly and importantly, serum IL-6 levels gradually decreased during long-term treatment, even though tocilizumab does not directly inhibit the synthesis of IL-6. This might be explained by the finding, in an animal model, that blockade of IL-6 signalling suppressed the induction of Th17 cells, which play a pathogenic role in the development of autoimmune diseases. In addition, we recently found that tocilizumab inhibited IL-6-induced RANK ligand expression on synovial cells obtained from RA patients, resulting in the inhibition of osteoclast formation. Tocilizumab also inhibited the gene expression of vascular endothelial growth factor, which causes neovascularisation that increases the supply of oxygen and nutrition to growing synovial tissues.
Conclusion: Clinical studies have demonstrated that targeting the IL-6 signalling pathway with tocilizumab could be an attractive and innovative therapeutic option for RA. It is highlighted that high efficacy was achieved consistently in several studies, and this adds to the evidence for the deep involvement of IL-6 in the pathogenesis of RA.
In addition, blockade of IL-6 signalling inhibited the induction of Th17 cells and inhibited angiogenesis and bone destruction.

Bile Acid Derivatives As BBB Modifiers

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Background: The aim of this study was to test the efficacy of the sodium salt 3*α*,7*α*-dihydroxy-12-oxo-5*β*-cholanate (MKC-Na) and methyl ester of 3*α*,7*α*-dihydroxy-12-oxo-5*β*-cholanate (MKC-Me) as a blood-brain barrier (BBB) permeator by examining its effect on quinine uptake into the central nervous system in rats. In our previous studies we have shown MKC-Na antidiabetic effect and potentiation effect on morphine analgesia.
Methods: Experiments were carried out on Wistar rats. Thirty minutes before injecting quinine to the right *a. axillaris*, the animals of the test group were given *s.c.* solution of MKC-Na in a dose of 2 mg/kg; the second group received *s..c* MKC-Me in a dose of 2 mg/kg; the animals of control group received *s.c.* saline solution. Animals of all groups were given quinine at a dose of 25 mg/kg by the retrograde bolus injection to the right *a. axillaris*. Before the decapitation the brain was washed with 5 ml of saline. The animals were decapitated 30, 60, 150, and 240 s after quinine injection. The brain was divided into: cerebrum, brain stem and cerebellum. After their weighing, the particular brain parts were homogenized. The quinine was extracted and analyzed in the homogenates by the method of Cram'er and Isaksson.
Results: Given with MKC-Na, quinine uptake by the cerebrum was increased 2.3 times, by brain stem 1.5 times and by cerebellum 1.7 times in the comparison to the control. Given with MKC-Me, quinine uptake by the cerebrum was decreased to 0.41, by brain stem to 0.39 and by cerebellum 0.32 in the comparison to the control.
Each value in the table represents the mean±SD (n=6)

Group	Control	MKC-Na	MKC-Me
CNS compartment	Cmax (pmol/g)	Cmax (pmol/g)	Cmax (pmol/g)
Cerebrum	3.39±0.31	7.80±0.65*	1.39±0.09*#
Brain stem	4.28±0.37	6.20±0.49*	2.44±0.15*#
Cerebellum	5.67±0.43	9.62±0.65*	3.11±0.25*#

* p<0.01 to the control;# p<0.01 to the MKC-Na group
Conclusions: The results indicate that MKC-Na is a potent enhancer of the BBB permeation, with the highest effect at the level of cerebrum. MKC-Me exibits an opposite effect in the test of quinine uptake and suggests a specific character of its action, depending on the bile acid derivative structure. The promotion of quinine uptake with MKC-Na may be the result of a mollecular agregates which does not occur with MKC-Me.

Challenges And Potential Solutions To Innovative Vaccine Development In Developing Countries

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Background: One of the barriers to meeting Millennium Development Goal Number 4 is access for large populations of developing countries to vaccines against their most serious and common diseases. Innovative vaccines have been more readily available at affordable prices once more producers enter the market, but this means that there will be a delay in access until these manufacturers can access the technology. Several solutions to enhance access have been proposed; none so far is optimal.
Methods: The factors relating to competition in the market and an affordable price were examined. Historical price data to countries were reviewed. Various explanations for lack of competition were also analyzed, including unequal access to financing, lack of optimal research and development capacity, barriers to technology transfer, issues in accessibility to intellectual property.
Results: Differences in vaccine scale up, know-how, GMP practices and regulatory oversight are decreasing between emerging suppliers and established multinationals. Developing country vaccine prices are at about the same levels for all manufacturers, but the level of vaccine development is lower for emerging suppliers who have few high priced markets to offset investments. There appear to be three major differences between multinational manufacturers and emerging suppliers:
(1) limited access to research results that lead to new vaccine constructs;
(2) barriers to vaccine technology development relating to blocking intellectual property;
(3) inability to spread the investments that would be incurred in addressing issues (1) and (2) over a large enough financial base.
In terms of pricing, experience with early adopting countries has shown that other factors may overcome the perceived pricing barrier: that is, willingness to pay appears not to correlate with country wealth.
Conclusions: Based on these analyses it appears that the interventions of the international community might be better directed to achieve vaccine access.

Enigmatic Eosinophil As Magic Bullet: Eosinophil-Induced Prognosis Improvement Of Solid Tumors Could Be Enabled By Their Vesicle-Mediated Barrier Permeability Induction

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Background: Eosinophils are multifunctional cells, which contain and produce many biologically active substances. Generally, eosinophilia is associated with parasitic infections or allergic disorders, while according to recent studies eosinophil infiltration is present also in target tissues of both physiological and pathological processes. With respect to solid tumors, the eosinophilic infiltration is associated with a better prognosis. In this review, the aim was to create a suitable hypothesis about this relationship.
Methods: The relevant abstracts of PubMed publications are used in this review. Apart from abstracts reporting data about the relationship between mentioned tumors and eosinophils, to create the hypothesis are used abstract which describe eosinophil functions or abilities in diverse physiologic or patho-physiologic situations.
Results: Reflecting on prognosis improvement in the case of solid tumors after eosinophilic infiltration of their capsules, it could be hypothesized that eosinophils are not tumoricidal per se; rather they can perforate such barriers through their vesicles' content, whereas the tumoricidal cytokines such as interleukin 4 (IL-4) fulfill the tumor necrosis. This scenario can be supported by the fact that IL-4 originated from macrophages and lymphocytes fails to mediate tumor necrosis in vitro conditions in absence of eosinophils. In analogy with solid tumors, the requirement of eosinophil-mediated increasing permeability among diverse biologic barriers and tissues may explain the eosinophils' introduction in capsules of cists, mucosal membrane of respiratory and gastroenteric systems, hemato-encephalic barrier, in embryos, as well as in bacterial and parasitic membranes.
Conclusions: In some situations such as solid tumors, rather than being multifunctional effectors per se, eosinophils, due to induction of target barrier dysfunction, may assure the host-required action, mediated by various kinds of leucocytes and their biologic effectors. Consequently, a better understanding of physiology and pathophysiology of this enigmatic cell will lead to new clinical strategies.

Selective Stimulation Of Human Natural Killer Cells Proliferation By Novel Fucosylated Acidic Glycan Drugs: Possible Therapeutic Use For Treating Cancer And Viral/Retroviral Infections

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Background: Definition of natural killer (NK) cells function is to kill cancer and viral/retroviral infected cells. Selective stimulation of different subsets of NK cells proliferation is the prerequisite for specific and effective target killing. The nature of the molecules responsible for such stimulation was not completely established and consequently therapy using NK route could not be valuably achieved. Our aims were: 1) To find a new class of drug molecules which can induce selective proliferation of different subsets of NK cell populations in humans. 2) To widen and complement therapeutic methods for treating cancer and viral/retroviral infections using NK cells as the "cellular magic bullet".
Methods: 1) Fucosylated acidic glycans isolated by chromatography and electrophoresis from sponges were sequenced using NMR and MS. 2) *Ex vivo* stimulation of human NK cells proliferation by these compounds was tested using peripheral blood mononuclear cells (PBMC) cultured in supplemented homologues serum for 1-3 weeks and measured by FACS analyses using antibodies markers for cell identification: CD3, TCR αβ, TCR γδ, CD4, CD8 - T cells; CD 16, CD56 - NK cells; CD20 - B cell; CD 14 monocytes. 3) *Ex vivo* killing of human tumor cells and viral infected cells with human NK cells were examined microscopically.
Results: 1) 33 novel fucosylated acidic glycan compounds were obtained. 2) Treatment of PBMC cultures with these compounds resulted in selective stimulation of proliferation of different NK cell subsets from 1-5% to 30-80%. Untreated controls remained at level of 1-5 %. No significant stimulation of B or T cells is observed. NK cells of all humans tested, from a variety of ethnic and racial groups could be significantly stimulated. 3) The obtained human NK cells showed massive and continuous killing of target human tumor or viral infected cells during five weeks of co-cultured period under condition of 1000 fold target cells excess.
Conclusions: 1) Novel class of 33 fucosylated acidic glycans compounds was isolated. 2) These drug compounds selectively stimulated proliferation of different subsets of human NK cells *in vitro*. 3) NK "cellular magic bullet" therapeutic effect for treatment of cancer and viral/retroviral infections is suggested to widen and complement existing treatments.
Authors' disclosure statement: This abstract contains novel and unpublished data.

Chemistry Of Nitrogen And Sulphur Based Compounds: An Approach Towards The Discovery Of Magic Bullets

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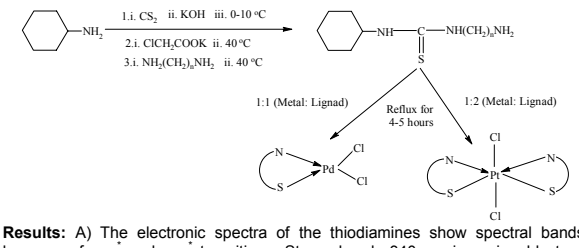
Background: The synthesis, physico-chemical investigation and biological studies of metal complexes of thiodimaine ligands are described which act as magic bullet for drug discovery in the area of bio-inorganic chemistry. Aims: 1) To synthesized platinum (IV) and palladium (II) complexes of the thiodiamines and characterized them by elemental analysis, IR, mass, electronic and ^{1}H NMR spectroscopic studies. 2) To screen the complexes for cytotoxicity, in vitro antifungal and in vitro antibacterial activities. In vitro antifungal and in vitro antibacterial studies were performed against fungal and bacterial strains, *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger and Escherichia coli* respectively.
Methods: The following scheme involves for the synthesis of thiodiamine ligand and their metal complexes



Results: A) The electronic spectra of the thiodiamines show spectral bands because of $\pi-\pi^*$ and $n-\pi^*$ transitions. Strong bands 340 nm is assignable to a combination of metal ligand charge transfer and d-d band. Strong bands at 2920-3250 cm^{-1} in thiodiamines are assigned due to –NH stretching vibrations of NH_2 and NH groups. The NH stretching of ligand has been found to shift to higher frequencies, the change being associated with coordination of terminal NH_2 nitrogen to the metal ion.
Conclusions: 1) The spectral studies indicate that the complexation takes place to the metal ion is through nitrogen and sulphur. 2) The in vitro antifungal activity of complexes as compared with standard drug Amphotericin B shows the minimum inhibitory concentrations (MICs) by microbroth dilution assays (MDA) and percent spore germination inhibition assays (PSGIA) are found to be 125-500 mg/mL. The in vitro antibacterial study of the complexes as compared with standard drug gentamycin shows the bacterial strains with the zone of inhibition were observed, 8-10 mm. The cytotoxic activity on primary adenocarcinoma show good activity at 100 mM solution as compared to standard drug cisplatin.

Ruthenium Complexes In Cancer Therapy: In Vitro And In Vivo Studies

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Background: Ruthenium-protein interaction leading to modulate the enzymes of cancer associated metabolic events is an evolving concept for anticancer ruthenium complexes. Lactate dehydrogenase (LDH; EC: 1.1.1.27) is found as the critical enzyme implicated in maintaining the tumor growth via executing 'Warburg effect' in cancerous cells. Although implication of M4-LDH in tumor development is quite evident (Koukourakis et al., 2003; Fantin et al., 2006), baring some exception (Niakan, 2001), reports are scanty on LDH inhibition dependent regression of tumors in vivo.
Methods: Several Ru(II) and Ru(III) polypyridyl complexes containing azolo/aryl-diazo pentane 2,4-dione, derivatives, 2,6-(2'-benzimidazolyl)-pyridine, 1,2,4-triazole derivative ,chalcones ,and flavones as co-ligands developed in our laboratory are tested against various tumor cell lines at Univ. of North Carolina USA for their cytotoxic and anti H IV activities [Bioorg. Med. Chem., 9, 1667,2001. Indian J. Experimental Biology, 42,660-666, 2004.J. Inorganic Biochemistry,99,1113-1118,2005]. To understand their mode of action on a preliminery level, their interaction with DNA (calf-Thymus) are monitored by UV.visible luminescence and NMR spectroscopic techniques. (New J. chem. (Royal Society Journal) 24, 505, 2000; Indian J. chem. 39A, 1295,2000).
Results: Ru(II) and Ru(III) polypyridyl complexes containing azolo/aryl-diazo pentane 2,4-dione, derivatives, 2,6-(2'-benzimidazolyl)-pyridine, 1,2,4-triazole derivative ,chalcones ,and flavones as co-ligands showed cytotoxic activity as Ic_{50} 0.8 and1.14I-µg/ml respectively against ovarian carcinoma and lung adeno carcinoma. This type of preliminary results suggested them as future drugs. We could recently describe LDH as a potential target of some metal complexes in vitro and in vivo including Ru (II) –Flavone and Ru (II) -CNEB .(Mishra et al., Indian J. Experimental Biology, 42,660-666, 2004. and Current Enzyme Inhibition 3, 243-353, 2007).
Conclusions: Currently we, have been exploring the mechanism of the action of such classes of drugs The results shows that Ru(II)-CNEB is able to decline M4-LDH significantly and to induce mitochondrial dysfunction – cytochrome c release-apoptotic pathway in DL cells in vivo. A concomitant reduction of DL cell viability with a significant improvement in the general living parameters of the tumor bearing mice, without producing any physiological and metabolic toxicity. The current findings will be presented which provides a biochemical basis to evaluate/screen anti tumor activity of related metal complexes on a number of tumor models.

Perfluorocarbons(PFC) As Universal Remedial Of Free Radical Homeostasis At The Wide Spectrum Of Various Metabolic Disorders

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Background: Routine biomedical applications for PFC and their emulsions include their use as a gas carriers, due to their ability transport and upload O_2 and CO_2. It's ability to dissolve great volumes of non polar gases (O_2 and CO_2) really dramatic. Oxygen solubility in PFC liquids is about 20–25 times greater than in either water or blood plasma under the same conditions. Also, it has been shown both in vitro and in vivo ability of PFC to modulate of physiologically significant pool of NO and other gases inorganic mediators. So, from our point of view, experience of use PFC may be extended for the account of wide spectrum metabolic disorders which caused or accompanied by free radical(FR) imbalance.
Methods: A. Experiments were performed on male Wistar rats (180g). Animals fed ethanol (EtOH) in drug dose (3.5 g/kg, intragastrically, 25% solution) twofold per day during 42 days. I group was sacrificed after 1 day, II-III groups were sacrificed after 7days following the last EtOH injection. During the last 7 days of experiment, animals of II group were two fold PF injected (1ml/100 gr. i.v.), III group were two fold NaCl 0.09% solution (injected 1ml/100 gr.i.v.). **B.** Experiments were performed on male Wistar rats (160g). Experimental animals fed EtOH in drug dose (4.0 g/kg, intragastrically, 25% solution) twofold per day during 60 days, but control animals were treated by NaCl 0.09% solution the same period. All animals were sacrificed after 12 hours after last injection. Group I was injection by EtOH only, group II was treated ethanol and last 3 weeks simultaneously EtOH and PF(1ml/100 gr. i.v., one fold per week). The activities of FR utilising enzymes, markers of alcohol injury of cells aminotransferases, gamma-glutamyltranspeptidase (GGTP), level of thiobarbituric acid-reactive substances (TBARS) and nitrite (NOx) were measured using Greiss reagent assay. **C.** The accessible literature about experience PFC application in clinic and experiments has been analysed and systematized.
Results: In studies **A**. and **B**. animals which fed EtOH, have strongly pronounced oxidative stress (FR processes activated, increased level of TBARS, greatly activated activity of GGTP, damaged hepatocyte membranes). PF application normalized level of lipid peroxidation and activity of GGTP, but strong reduced both liver and plasma NOx content. In other words PF can effective correct metabolic changes in the liver caused by EtOH administration. C. Experience PFC application were shown similar immunomodulating, antioxidant, membrane-stabilizing and disintoxicating properties at the wide spectrum disorders.
Conclusions: Results suggest that the PF application at various metabolic disorders (cardiology, oncology, ophthalmology etc.) had one general basis, founded on modulation level of FR, restoration cell membranes and may be a conceptually new pharmacological tool for various metabolic complications associated with free radical imbalance. In the work were discussed main mechanisms of the obtained findings.

Aptamers As Magic Bullets And Delivery Vehicles In Disease Imaging And Therapy

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Background: The term of 'magic bullet', coined so long ago, would be most appropriate for these novel targeting modalities termed aptamers. Aptamers are nucleic acid in nature, and although they are larger than traditional chemotherapy agents (~10kDa), they are smaller than antibodies and other protein-based biologics. Furthermore, they have high affinity and specificity for their target, significantly higher than that of small molecules or peptide and even antibody therapeutics.
Method: Aptamers are usually selected by the SELEX methodology and we have used anything between 1 and 10 round of selection and amplification to isolate high affinity and specificity ligands. These are subsequently modified to ensure resistance to nuclease degradation and their target affinity is assessed by SPR, spectroscopic and immunological and immunohistochemical techniques, whilst the pharmacokinetic and tumour penetration properties have been assessed in experimental model systems.
Results: Aptamers are ideal magic bullets that can travel throughout the body with minimal immunogenic or toxic effects, without entering cells that are not carrying the aptamers' cell-surface receptor target, bind specifically and inhibit the action of their target. This has led to the success of aptamers in the market as antiangiogenic agents with aptamers against VEGF for the treatment of macular degeneration, and as inhibitors of nucleolin in clinical trials against cancer and our own aptamers in preclinical studies with excellent tumour penetration properties. Furthermore, where the cell-surface receptor is not part of a vital pathway and its blockade does not lead to cell kill, aptamers have sufficient mass and relevant pharmacokinetic properties to act as carriers of toxins, chemotherapy agents or radionuclides to exert their therapeutic effects. Finally, this therapeutic action of aptamers is complemented with their potential as diagnostic and imaging agents, when their carrier allows distant recognition, such as gamma emitters, contrast agents, NIR emitters or fluorescent agents. An overview of the aptamers' characteristics and applications in disease diagnosis and therapy, examplified with novel results from own research and that of other groups will be presented.

Toll-Like Receptor (TLR) Agonists And The Induction Of The Innate Immune Response

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Background: TLRs are pattern recognition sensors that induce cellular immune responses to the systemic presence of a variety of microbes. TLR agonists are in active development as pharmaceuticals for a variety of indications. The most advanced in clinical development are –CpG- (TLR9), LPS (TLR4), and dsRNA (TLR3) agonists. Nine of the ten human TLRs utilize a common intracellular signaling pathway. TLR3 employs a unique pathway that may be responsible in part for its relative lack of toxic inflammatory responses. TLR3 ligands have been demonstrated to have broad anti-viral, immunomodulatory, and anti-proliferative responses in a wide spectrum of pre-clinical studies. The parent TLR3 agonist, Poly I:Poly C, is not limited to TLR3 activation and is associated with a variety of toxic responses in humans. The analog, Poly I:Poly $C_{12}U$, is active only as a ligand for TLR3 and is not associated with dose limiting toxicities which facilitates clinical utilization for a variety of indications.
Methods: A variety of studies have demonstrated the utility of Poly I:Poly $C_{12}U$ as an inducer of innate immune responses in the prophylaxis and treatment of human diseases. These include:1) Phase 2 and 3 clinical trials for chronic fatigue syndrome (CFS), 2) utilization as an immune enhancer for avian HPIV vaccines, and 3) treatment of human renal cell carcinoma.
Results: 1) Double-blind, placebo-controlled, Phase 2 and 3 clinical trials with Poly I:Poly $C_{12}U$ have demonstrated efficacy in primary end-points and a variety of secondary end-points in well defined CFS. These include exercise tolerance, decrease in drug usage for symptoms of CFS, and a decrease in physician and patient assessments of disease symptoms. Poly I:Poly $C_{12}U$ was well tolerated; 2) The intranasal administration of Poly I:Poly $C_{12}U$ with a Japanese seasonal influenza vaccine in mice provided protection against challenge from the 3 viruses represented in the vaccine as well as challenge with avian H5N1 HPIVs; 3) An open-label study in human renal cell carcinoma (Elson Risk Group =3) was compared to historical controls stratified to Risk Groups 1-5 over a 2 year observation period. Poly I:Poly $C_{12}U$ provided significantly improved survival compared to Risk Groups 2- 5.
Conclusions: Poly I:Poly $C_{12}U$ is a well-tolerated and active TLR3 agonist under current clinical development for a variety of indications.
Authors' disclosure statement: Studies on Poly I:Poly $C_{12}U$ have been funded by Hemispherx Biopharma, by contract through NIH (NIAID/ NCI), or by the NIID (Japan). WMM is an independent member of the BOD of Hemispherx Biopharma.

Helicobacter Cysteine-Rich Proteins A And -C, Two Novel Signalling Molecules Form The Family Of Sel1-Like Repeat Proteins Involved In The Modulation Of Pathogen/Host Interactions

MITTL P

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The spiral-shaped gram-negative ε-proteobacterium H. pylori settles in the gastric lumen of primates and higher vertebrates and shows an exceptional adaptation to its ecological niche, resulting in long-lasting infections of the gastric mucosa. The persistence of H. pylori infections is most likely a consequence of its ability to modulate the innate immune system of the host. To identify novel virulence factors we initiated an inverse genetics approach, focusing on a family of secreted proteins that are specific for å-proteobacteria. Due to the high content of disulfide bridges this family was designated Helicobacter cysteine-rich proteins (Hcp). The crystal structures of HcpB and -C served as the prototype structures for the large family of Sel1-like repeat proteins that participate as protein/protein-interaction modules in signaling pathways of bacteria and eukaryotes but not in archea.
Expression of Hcps under in vivo conditions was confirmed by the detection of elevated anti-Hcp IgG titres in H. pylori positive patients. Since Hcp knock-out experiments suggested that Hcps might be important for the interaction between the bacterium and its host, we investigated the effects of recombinant Hcps on mammalian leukocytes. HcpA elicits the release of cytokines from naive mouse splenocytes and also affects the morphology and adherence of monocytes in vitro.
The sequence analysis of Hcp homologues from a large collection of H. pylori strains uncovered amino acid positions that seem to be important for the functionality of Hcps and for the adaptation to different host populations.
Thus, Hcp serve as bacterial signaling molecule that modulate important properties of mammalian immune cells. To gain deeper insights into the adaptation of H. pylori to different host populations we are currently working towards the analysis of the Hcp signal transduction mechanisms. Recent progress in the identification and validation of eukaryotic Hcp receptor molecules will be discussed.

Cadmium Stress Associated Protein Mediated Resistance to *Fusarium* Infection in Wheat

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Background: Plants do not have a defined immune system directly comparable to that of animals. However, plants respond to pathogen attack by a variety of biochemical means known as defense responses. Particularly, during fungal infection, plants synthesize low molecular mass inhibitory compounds such as, phytoalexins and accumulate pathogen-related (PR) proteins. In many plants, enhanced disease resistance is accompanied by the activation of genes encoding pathogen-related (PR) proteins [30]. Recently, the defense-role of sugarcane glycoproteins against the smut disease in sugarcane caused by *Ustilago scitaninea* was demonstrated. Similarly, there has been increasing evidence for direct influences of strobilurins, a fungicide, on plant defense physiology, which suggest that in addition to their fungicidal activity, strobilurin also enhances the capability of plants to ward off viral and bacterial pathogens. The anti fungal activity of a strobilurin compound, F-500 (Pyraclostrobin) has been tested in tobacco plants which, enhanced plant resistance against tobacco mosaic virus (TMV) and bacterial pathogen, *Pseudomonas syringae* pv *tabaci* . Some chemicals and metal ions are anti fungal in that the treatment of seeds prevents or arrests fungal infection to plants. Cadmium salts are usually used as antifungal . However, cadmium is also phytotoxic, athigh concentration it retards plant growth and development . Exposure of plants to heavy metals like Cd2+ induces production of phytochelatin and some stress associated proteins,SAP. However, no report has been found on Cd2+ pre-exposure imparting resistance to fungal specification .In this communication, we report, for the first time, on a novel mode of antifungal activity of $CdCl_2$ in that a pre-exposure of wheat seedlings to a mild dose of CdCl2 imparts immunity to plants against Fusarium infection.Aims:1) Structural characterization of cadmium associated stress protein(CSAP) induced in wheat with the treatment of a low dose $CdCl_2$ .2)To assess the possible direct and/or indirect role of glutathione(GSH) against fungal infection in wheat apart from its heavy metal detoxification activity.
Method: Surface sterilized wheat (*Triticum aestivum* L.) seeds(100), treated with 0.01 % HgCl2 (m/v) for 10 min and washed thrice in sterile distilled water, were treated with50 µM CdCl2 for 48 h at room temperature (28 ± 2 °C).The seeds were allowed to germinate on filter paper saturated with sterile distilled water. An equal number of seeds without Cd2+ treatment served as control. The Cd content of the control and the treated seedlings were estimated by atomic absorption spectrometry. Seven days old untreated (control) and CdCl2 pre-exposed seedlings with a well developed root system were grown in test tubes containing liquid MS (1/10 strength) medium supplemented with 4-day-old freshly cultured *F. oxysporum* (1x106) inoculums. The mild dose Cd2+ pre-exposed seedlings maintained in tubes containing *F. oxysporum* with MS basal medium were considered as test plants while the only Cd2+-exposed seedlings served as comparative control. Seedlings without Cd2+ pre-exposure (control) and seedlings grown in only *F. oxysporum* inoculums with MS basal medium were also maintained for comparison .A total 48 number of seedlings were used per each set of experiment and repeated for three times. All test and control plants were incubated at 26 °C ± 2 °C .Two seedlings per tubes were considered for better observation. The tolerance of the plant assessed was based on the survival and growth of the seedlings under stressed conditions. Growth and survival data were recorded for a period of another seven days.
Fourteen days old germinated seedlings (500 mg) were removed from the seeds and crushed with 10mM sodium phosphate buffer (pH 7.55). Samples were then centrifuged at10000 rpm (2,500 g) using a centrifuge for 10 min. (Remi). The supernatant containing protein was collected and pellet was washed, re-centrifuged and then discarded. Protein was estimated according to Lowry *et al*. Protein samples were dialyzed against the same phosphate buffer with two changes for 24h each and finally concentrated by using polyethylene-glycol (PEG-60).
Extracted protein was characterized on a 10% SDS-PAGE and the molecular weight of the particular protein band of control (without treatment), Fusarium treated, Cd2+ (50µM)-exposed and co-stressed (50µM Cd2+ pre-exposed and then infected with *F.oxysporum*) seedling was determined from their Rf values. The particular protein band of interest, which was expressed dominantly due to stress induction, was cut out from the particular gels and the protein was electro-eluted in a Bio-rad (Electro-eluter) apparatus, using the electro-elution buffer (Tris 25mM, glycine 192mM and SDS 0.1%, pH 8.3) at a constant current of 8-10mA/glass tube for 5h. The eluted protein was collected, and concentration was estimated and again ran on a 10% SDS-PAGE to establish its homogeneity. Further in-gel triptic digestion and liquid chromatography mass spectrometry (LC-MS/MS) analysis was done. The purified protein was dissolved in water at a concentration of 80 µg cm-3. The N-terminal sequence ofCSAP was obtained by automated Edman degradation,followed by HPLC and UV detection (Edman and Begg1967), using a *PPSQ-21A* protein sequencer (*Shimadzu*,Kyoto, Japan). Searches for sequence similarity were performed using *Blast P* databases.

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Mechanism Of Oxidative DNA Damage And Apoptosis Induced By Doxorubicin Through Generation Of Reactive Oxygen Species

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Background: The anticancer mechanism of doxorubicin (DOX), an anthracycline antibiotic, is believed to involve DNA damage and apoptosis through topoisomerase II inhibition and reactive oxygen species (ROS). However, the precise mechanism of DNA damage and apoptosis induced by DOX remains to be clarified. We investigated the mechanism of DNA damage and apoptosis induced by DOX.
Methods: We used human leukemia cell line HL-60 and its H_2O_2-resistant HP100 cells. Apoptosis induced by DOX was detected by DNA ladder formation. Detection of peroxide and □□m in cells treated with DOX was analyzed on a flow cytometer. Measurement of caspase-3 activity was used by DEVD-AFC, a caspase-3 synthetic substrate. Measurement of 8-oxodG, a marker of oxidative DNA damage, was used by HPLC-ECD. In addition, we analyzed DOX-induced DNA damage, using ^{32}P-labeled DNA fragments.
Results: DOX-induced DNA ladder formation could be detected earlier in HL-60 cells than in HP100 cells, suggesting the involvement of H_2O_2-mediated pathways in apoptosis. Flow cytometry revealed that H_2O_2 formation preceded the increase in □□m and caspase-3 activation. Poly (ADP-ribose) polymerase (PARP) and NAD(P)H oxidase inhibitors prevented DOX-induced DNA ladder formation in HL-60 cells. Moreover, DOX significantly induced formation of 8-oxodG, in HL-60 cells at 1 h, but not in HP100 cells. DOX-induced apoptosis was mainly initiated by oxidative DNA damage in comparison with the ability of other topoisomerase inhibitors to cause DNA cleavage and apoptosis. Moreover, DOX caused site-specific oxidative DNA damage in the presence of copper(II), which may contribute to apoptosis.
Conclusions: These results suggest that the critical apoptotic trigger of DOX is considered to be oxidative DNA damage by the DOX-induced direct H_2O_2 generation, although DOX-induced apoptosis may involve topoisomerase II inhibition. This oxidative DNA damage causes indirect H_2O_2 generation through PARP and NAD(P)H oxidase activation, leading to the □□m increase and subsequent caspase-3 activation in DOX-induced apoptosis.

Chitosan-Ca-Alginate Microparticles As Carriers For Colon Delivery Of 5-Aminosalicylic Acid After Peroral Administration

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Background: 5-aminosalicylic acid (5-ASA) is widely used for its local effects in the treatment of inflammatory bowel diseases (IBD). The optimum oral 5-ASA delivery system requires technology that protects the drug during microencapsulation and distribution in the stomach and small intestine. For these requirements, a new chitosan-Ca-alginate microparticulated colon drug delivery system was prepared and characterized.
Methods: Microparticles were prepared by spray-drying technique. SEM was used to analyze particles' size and surface morphology and for polymers localization, CLSM was used. Polymers interactions were evaluated using X-ray and DSC, while for 5-ASA stability, ^{1}HNMR spectra in H2O/D2O were recorded. Influence of formulation variables on microparticles' properties was evaluated and for multiple response optimization, mixed 2- and 3-level fractional factorial design was used. Drug release studies in different pHs and in rat cecal content were performed. Biodistribution and therapeutic effect of 5-ASA were tested in rats in which colonic inflammation was induced.
Results: Particles with zeta potential between -34 and 10 *mV* were obtained, with mean diameter less than 15 □m, calcium content between 2.5 and 5% and encapsulation efficacy between 50 and 70%. Chitosan was localised dominantly in the particle wall, while for alginate, homogeneous distribution throughout the particles was observed. Thermograms and X-ray diffractograms indicated molecularly dispersed drug within the particles. Stability of 5-ASA during microencapsulation and in different pHs was also confirmed. Anomalous (non-Fickian) transport mechanism in 5-ASA release was determined, controlled by polymer relaxation, erosion and degradation. Biodistribution and efficacy studies in the animal model of inflammation confirmed the controlled release and colon specific delivery of 5-ASA.
Conclusion: The described 5-ASA loaded chitosan-Ca-alginate microparticulated system might be successfully used for clinical treatment of IBD.

Swaziland Demographic and Health Survey 2006–2007 with Special Focus on the Prevalence Of HIV

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Background: The 2006-07 Swaziland Demographic and Health Survey (SDHS) was a national-level sample survey which was implemented by the Central Statistical Office (CSO) at the request of the Ministry of Health and Social Welfare (MOHSW). The principal objective of the survey was to provide up-to-date information on prevalence of HIV among other things. The aim was to identify behaviours that protect or predispose the population to HIV infection, examine social, economic, and cultural determinants of HIV, and determine HIV prevalence among males, females and children age 2 years and older.
Methodology: Children age 2 to 14 years and older adults age 50 years and above in half the households selected for the SDHS sample were eligible for the HIV testing. Over 13.000 women, men and children living in the households selected for the survey were test. HIV prevalence data was obtained from finger stick dried blood spots voluntarily provided by the women and men 18 years and over who were interviewed and children 2-14 years whose parents gave consent and were members of households interviewed in the survey. Children age 15-17 were also asked to provide consent in addition to obtaining parental consent.
The data was processed by office editing, coding of open-ended questions, data entry, double-entry verification, and resolving inconsistencies found by computer programmes developed for the SDHS. The SDHS data entry and editing programmes used CSPro, a computer software package specifically designed for processing survey data such as that produced by DHS surveys.
Results: The results indicate that 26 % of adults are HIV infected; 31% of women and 20% of men are infected. Prevalence is higher in urban than rural areas, 37% and 29% respectfully. Almost one in two women age 25-29 is HIV-positive. Prevalence is highest among those who are divorced, separated, and widowed. Prevalence increases with number of lifetime partners and 17% of couples were discordant
Conclusion: This information provide data to assist policymakers and programme implementers to monitor and evaluate existing programmes and to design new strategies for demographic, social and health policies in Swaziland. The survey also provides data to monitor the country's achievement towards the Millennium Development Goals.

Antimicrobial Resistance In Major Pathogens Of Surgical Site Infection In Iran Hospitals

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Objective: Surgical site infections are a significant problem which limits the potential benefits of surgical interventions. The impact on hospital costs and postoperative length of stay is considerable. The increased occurrence of antimicrobial-resistant microorganism is a major medical concern. We determine the resistance to antibiotics in major pathogens isolated from surgical site infection in our hospitals in Isfahan, Iran.
Methods: Antimicrobal susceptibility of bacterial isolated from surgical site infection in two university hospitals from 2005 to 2006 was monitored by Epsilometer test E (E-test®;AB BIODISK Co. Sweden).Data was analyzed by Whonet 5.3 software. Quality control was tested by *E.coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213. Results: The most frequent pathogens were *Staphylococcus aureus*(36.8%) followed by *Klebsiella spp.*(17.1%), *E. coli* (15.1%), *Pseudomonas aeruginosa*, (12.5%) and coagulase negative *Staphyloccoci*(8.6%). The susceptibility rates *Staphylococcus aureus* to oxacillin with minimum inhibitory concentration rang(0.094-256µg/ml) were 32.3% , vancomycin with MIC rang(0.38-32µg/ml) were 94.8% and clindomycin with MIC rang(0.047-256µg/ml) were 41.7%%. In gram negative bacteria the most active antibiotic was imipenem and meropenem. The susceptibility rates of *Klebsiella* to imipenem,meropenem, cefepime, ciprofloxacin, ceftazidime, and ceftriaxone were 94.9%,86.1%,23.5%,20%. The susceptibility rates of *E.coli* to imipenem,meropenem, cefepime, ciprofloxacin, ceftazidime, and ceftriaxone were 95.8%, 92.2%, 36.9%, 50%, 32.5%, 28.6%.
Conclusion: In Iran, like other countries antimicrobial resistance is a serious clinical problem among healthcare facilities. Each healthcare facility should have an antimicrobial use committee. Surveillance of antimicrobial resistance should be improved and antimicrobial restriction is also an important intervention.

The Psychological Outcome Of Male Constitutional Delay Short Stature

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Background: Growth is said to be the defining feature of childhood, with genetic and environmental factors influencing the rate of growth and the final stature. Studies have demonstrated that idiopathic short stature, familial short stature and constitutional delay of growth male children often respond to growth treatment: Mental health outcome studies of male short stature have varied but these studies have been difficult to compare due to differences in cohort age groups and differences in assessment tools. However past studies have investigated psychological outcome as being dependent on height (short versus tall).
Methods: A novel approach was devised to measure how adult male final height was dependent upon the mental health status for those treated (Rx+, n=27) and untreated (Rx-, n=21) with growth promoting products as children defined as having constitutional delay. The respondents were assessed by the SCL-90-R psychological distress and psychopathology instrument and allocated into either a clinical, distressed group, or a non-clinical, non-distressed group.The Rx+ respondents had received as children (1) oral oxandrolone or (2) intramuscular injections of testosterone esters; other oral preparations may have been used but growth hormone was not used.
Results: (1) The main finding was that there was a significant relationship between psychological distress and height outcome for the group (Rx+ Rx-) in psychological distress and the group (Rx+ Rx-) not in psychological distress. Chi-Square p<0.03. (2) The average gain in height from treatment in the non-psychologically distressed group was 1.8 cm and the for psychologically distressed 1.4 cm.
Conclusion(s): (1) Those not in psychological distress were height advantaged. (2) Had the psychological state not been controlled then the outcome findings would have been decidedly confounded. (3) The height improvement for the treated cohort can be compared with similar modest increases using recombinant growth hormone for growth promotion in other studies. (4) When male children present for investigation and possible treatment for growth promotion they should be assessed for their mental health status as this may well be a predictive factor on adult height outcome which further longitudinal studies may resolve.

Phase III Study Of Combined Neoadjuvant Chemotherapy And Letrozol In Locally Advanced Breast Cancer

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Background: Despite early detection of breast cancer, locally advanced breast carcinomas account for a remarkable fraction of all breast carcinomas. The optimal management for these patients remains a major therapeutic challenge. Neoadjuvant chemotherapy is now considered the standard of care for these patients. Adjuvant and neoadjuvant endocrine therapy with aromatase inhibitors has an established role in the postmenopausal women with hormone receptor-positive invasive breast cancer. This two arm randomized clinical study aimed to evaluate the efficacy and safety of the combined neoadjuvant chemotherapy and letrozol in postmenopausal women with locally advanced breast carcinoma.
Methods: Fifty eligible women with pathologically proven locally advanced breast cancer were enrolled. Chemotherapy consisted of a median 3 cycles (range 2-4 cycles) of CAF regimen (Cyclophosphamide 600 mg/m^2, doxorubicin 60 mg/m^2, 5-FU 600 mg/m^2) every three weeks. Patients in study arm (n=25) received daily letrozol 2.5 mg combined with neoadjuvant chemotherapy for a median time of nine weeks (range 6-12 weeks). Patients in control arm (n=25) received neoadjuvant chemotherapy alone for same time. All patients underwent modified radical mastectomy 3 weeks after the last cycle of chemotherapy. Pathologic response rate was the primary end-point of the study.
Results: Five (20%) of patients in study group and three (12%) of patients in control arm had inflammatory breast cancer. Overall pathologic response rate was 96% and 68% in study and control arm respectively (p=0.023). Complete pathologic response rate was 28% and 4% in study and control arm respectively (p=0.049). All patients had a resectable disease after neoadjuvant treatments. There was no difference in terms of treatment-related toxicity rates between two arms. Hematologic toxicity was the most frequent treatment-related toxicity in both arms.
Conclusions: The present study suggests that the addition of concurrent letrozol to the neoadjuvant chemotherapy significantly improves the response rates. Whether this approach confers a survival advantage remains to be determined.

Magnetic Nanoparticles For Controlled Delivery Of Methotrexate

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Background: Cancer is a major cause for morbidity and mortality in industrialized countries. For patients with advanced disease, chemotherapy based on methotrexate is currently the mainstay of treatment. We have developed a novel water-dispersible oleic acid (OA)-Pluronic-coated iron oxide magnetic nanoparticle formulation that can be loaded easily with high doses of water insoluble anticancer agents.
Methods: Nanoparticles of iron oxide was synthesized by mixing Aqueous solutions of 0.1 M Fe(III) (30 mL) and 0.1 M Fe(II) (15 mL) and dropwise adding of 3 mL of 5 M ammonia solution over 1 min under continuous stirring on a magnetic stirrer plate. The stirring continued for 20 min under a nitrogen-gas atmosphere. The iron oxide nanoparticle yield, determined by weighing of the lyophilized sample of the preparation. Formulations of iron oxide nanoparticles were developed, first by optimizing the amount of OA required to coat iron oxide nanoparticles completely, and then by optimizing the amount of pluronic required to form an aqueous dispersion of OA coated nanoparticles. To study the effect of OA, formulations with different weight ratios of OA to iron oxide nanoparticles were prepared. For this purpose, OA was added from 60-240 mg corresponding to same weight of iron oxide nanoparticles. After the coating of nanoparticle with pluronic, methotreate was loaded to particles.
Results: The loading efficiency was more than 90 % in optimized condition. Neither the formulation components nor the drug loading affected the magnetic properties of the core iron oxide nanoparticles. The release pattern was uniform in water for 8 hrs under in vitro conditions.
Conclusion: This system could be used to deliver anticancer drug methotrexate to the tumor site by using an appropriate external magnetic field. Based on the release pattern it would be practical to target the drug in higher concentration in tumor site than the other sites of the body.

Trial Of Lamivudine In Hepatitis B Surface Antigen Carriers With Persistence Hepatitis B Core IgM Antibody

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Background: The persistence of hepatitis B core IgM antibody in hepatitis B surface antigen carrier is a risk factor with hidden danger and forecast existence of liver damage. A trial of lamivudine in such subset of carriers was carried on for the first time in this study.
Methods: A total of 62 hepatitis B surface antigen with hepatitis e antibody individuals (age range, 25-45 years) with persistence hepatitis B core IgM antibody were randomized to receive either 100 mg lamivudine (32/62) or placebo (30/62) daily for 6 months.
Results: Among lamivudine group, hepatitis B core IgM antibody seroclearance achievement rate was 81.3% and HBsAg seroconversion rate was 9.4 % compared to 6.3% and 3.3% in placebo group respectively. A number of adverse clinical events were observed, but were of mild nature and tolerable by the participants who completed the study.
Conclusion: Trial of lamivudine in this subset of hepatitis B surface antigen carriers proven to be safe and efficacious. More studies are needed prior to recommending the drug for routine use on selected HBV carriers.

Targeted Therapeutics: From Magic Bullets To Multifunctional Nanoparticles

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Background: The first 'magic bullet', arsphenamine, a synthetic organic arsenic compound, was discovered by Paul Ehrlich in 1909 and proved to be the best cure for syphilis at that time. The world of medicine has changed unimaginably since then, with the discovery of DNA as the genetic material, the evolution of biotechnology, and more recently the promising new dimensions of nanobiotechnology. This presentation intends to review how the state-of-the-art in therapeutics, irrespective of whether they are small molecular drugs or genes, deploy the multifunctional nanoparticles to achieve specific targeting of drugs.
Methods: A plethora of technological advances in genomics, including the sequencing of the entire human genome with its revelation of disease-related genes and the discovery of small interfering RNAs and micro RNAs have led to novel targets. Also, substantial progress has been made on the nanotechnology and nanoscience front in applying new knowledge to targeted gene and drug delivery strategies.
Results: A diverse array of human-friendly polymeric biomaterials has been developed into multifunctional nanoparticles, which can not only carry the drug payload but also specific targeting and imaging moieties. A myriad of cell targeting approaches have been tested including not only antibodies, but also small molecular ligands, peptides and other biologics. Examples of successful applications of combined genomics and nanotechnology will be presented.
Conclusion: Thus, multifunctional nanoparticles provide a platform and new vistas in cell-specific targeting of drugs and biologics and represents rebirth of 'magic bullet'.
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Methemoglobin As The Biomarker Of Environmental Oxidants And Precursor Of Adverse Effects Of Oxidative Sress On Mother And Fetus - Reasons For Its Early Detection And Therapy

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Background: Methemoglobin (MetHb) and its catabolic products are prooxidant. In the first blood circulation stage, the inhalation of nitrogen oxides transforms the hemoglobin into its pathological MetHb. Methemoglobinemia symptoms in "maternal preeclampsia" are also common in severe anemia, preeclampsia and eclampsia, suggesting them to be a precursor for these conditions. The oxidants crossing the placental barrier cause "fetal preeclampsia". The levels of MetHb and its catabolic products as bilirubin-biliverdin, CO and toxic Fe (III) with paramagnetic nature probably go over to BBB and provoke adverse effects on brain development.
Objective: The objective is to confirm the anatomo-pathological and genetic alterations that underlie sudden unexpected and unexplained perinatal and infant death and their correlation with environmental factors.
Methods and results: The MetHb level was determined from the samples that were taken three times, with a one-month pause between each test, for each pregnant woman (N=122) in the exposure period of power plant operation and in the control period when the power plant was closed (N=138). The significant positive correlation between the level of MetHb and the daily ground level concentration of SO_2 was found ($r=0.72$, $p<0.01$). The reproductive loss was significant between the »control« (N=4) and »exposure« periods (N=10) ($p=0.0369$) and the frequency of stillbirths with the amount of MetHb >1.5 g/L in the exposure period was also statistically significant ($p=0.0336$). The test of chromosome aberration (SCI) was not significant in newborns whose mothers' MetHb level was >1.5 g/L (N=36). We observed them by collecting data from hospitals, preschool and school services at health centers until they were eighteen years of age, and we found that the incidences of neonatal jaundice ($p=0.034$), later heart murmur ($p=0.011$) and dyslalia and learning memory mpairments ($p=0.002$) were significantly higher than in children born by control mothers (n=19).
Conclusion: The MetHb level is a useful biomarker but also has a degree of predictive validity. Our current research intends to study the brain morphological aspects of the development of both the autonomic nervous system and the cardiac conduction system and to individuate the environmental interactions resulting in the perinatal unexplained death, as well as to help find therapy, especially in underdeveloped countries with high maternal and perinatal morbidity and mortality.

Plant Alkamides Bioactive Molecules On A Wide Range Of Organisms

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Along human history, different civilizations have traditionally used many plant species both as herbal medicines and as stimulants. The molecular activities that are responsible for these effects typically result from metabolites present in plant tissues. In many of these herbal remedies alkamides are present as bioactive components.
In recent years, it has become apparent that there are novel signaling molecules, such as N-acylethanolamides (NAEs), alkamides, glutamate and nitric oxide, which might play important roles in the regulation of morphogenetic and adaptive processes. Recent work with *A. thaliana* seedlings has provided evidence of the potent biological activities of some alkamides, such as affinin widest distributed alkamide in plants, and NAEs in seedling growth and root development.
In animals, NAEs acting as endogenous signaling molecules, and alkamides interact with cannabinoid receptors, which are coupled to signal. Hence, there is a possibility that cannabinoid signaling represents an evolutionary conserved pathway that modulates cellular and physiological processes in eukaryotes. In plants the formation of NAEs, structurally related to alkamides and to anandamide, was initially associated with germination and responses to pathogen attack.
Studies in our lab showed that alkamides in lower concentrations, but not NAEs, greatly stimulate roots and aerial parts development, however both groups of compounds in higher concentrations inhibit plants development. Two affinin-derived alkamides (*N*-isobutyl-2*E*-decenamide and *N*-isobutyl-decanamide) were found to be even more active than affinin in stimulating cell responses.
Unsaturated alkamides have been shown to be fungistatic and bacteriostatic. Additionally when *N*-isobutyl-decanamide is applied to *A. thaliana* seedlings cultured *in vitro*, an increased expression of defense response genes occurred in parallel with accumulation of salicylic acid and jasmonic acid.

Cell Cycle Kinases As Molecular Targets In Anticancer Therapy

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Methods: Small molecule kinase inhibitors were identified by screening a proprietary kinase targeted library of approximately 50.000 compounds using recombinant kinases in a biochemical screening format. After reconfirmation, hits were screened for cellular activity and mechanism of action *in vitro* and *in vivo*. Efficacy and PK/PD modeling of lead compounds were assessed in different tumor animal models. Toxicity was assessed in primary and secondary non-rodent species.
Results: Upon screening of our chemical library for Aurora, Polo and CDC-7 kinases we have identified potent and selective hits. These hits were optimized and SARs were developed. Upon lead optimization, small molecules were identified for all targets and were found to be highly efficient *in vivo* and are well tolerated. The most advanced compound, PHA-739358, was the first Aurora kinase inhibitor to enter clinical trials and is currently in phase II. An additional clinical opportunity is based on its cross-reactivity with wild-type and T315I mutant BCR-Abl, which will be shown and discussed.
Conclusions: Small molecules inhibiting different cell cycle kinases have potent anti-tumor activity, however the cellular responses and mechanisms of action are distinct for inhibitors of Aurora, Polo and CDC-7 kinases. This difference might be important for treatment of different indications or subsets of patients. It is expected that small molecules inhibiting these targets will add to the future armory of available anti-cancer agents.
Authors' disclosure statement
All authors are full-time employees of Nerviano Medical Sciences Srl.

Expression Of MAGE-A12 In Oral Squamous Cell Carcinoma

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Background: Melanoma associated-A antigens (MAGE-A) are silent in normal tissues except testis. However, they are activated in a variety of different tumors. Thus, their expression is highly specific to cancer cells. Reverse transcription-nested polymerase chain reaction (RT-nPCR) is a highly sensitive technique that has been used successfully for the detection ofMAGE genes in tissue samples. Aim: To analyze the expression rate of MAGE-A12 in oral squamous cell carcinoma (OSCC) using a high sensitive RT-nPCR.
Methods: Total of 57 tissue samples obtained from patients with OSCC and 20 normal oral mucosal (NOM) probes of otherwise healthy volunteers were included to this study.
Results: No expression of MAGE-A12 was observed in the non-neoplastic NOM tissues. MAGE-A12 was expressed in 49.1% of the investigated tumor samples. The correlation between malignant lesion and MAGE-A12 detection was significant ($p < 0.001$).
Conclusions: 1) Results of this study may indicate MAGE-A12 as a useful additional diagnostic marker especially for the early detection of OSCC distinguishing neoplastic transformation and detection of occult and/or rare disseminated cancer cells. 2) MAGE-A12 expression in OSCC may also determine a new immunotherapeutic target and might be warranted to develop vaccine for OSCC.

Trans-Cinnamaldehyde From *Cinnamomum Zeylanicum* Bark Essential Oil Reduces The Clindamycin Resistance Of Clostridium Difficile *In Vitro*

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Background: Therapy with antimicrobial drugs, such as clindamycin, that perturb the intestinal flora but fail to inhibit growth of other microorganisms can permit the proliferation of *Clostridium difficile* and the elaboration of exotoxin. Therefore, there has been increasing interest in the use of inhibitors of antibiotic resistance for use in combination therapy. The essential oil of *Cinnamomum zeylanicum* bark enhanced the bactericidal activity of clindamycin and decreased the minimum inhibitory concentration of clindamycin required for a toxicogenic strain of *Clostridium difficile*.
Methods: Thin-layer chromatography (TLC) analysis of the essential oil separated a fraction (R_f = 0.54) that was the most effective at enhancing the clindamycin antimicrobial activity. Using gas liquid chromatography and known standards, the active fraction was identified as trans-cinnamaldehyde (3-phenyl-2-Propenal). Combinations of clindamycin and trans-cinnamaldehyde were tested to determine the fractional inhibitory concentration (FIC) index by conventional checkerboard titration. The FIC index for *Clostridium difficile* was found to be 0.312, which confirmed the synergistic actions of clindamycin and trans-innamaldehyde.
Results: The presence of 20 µg/mL of trans-cinnamaldehyde decreased the MIC of clindamycin for *Clostridium difficile* sixteen-fold, from 4.0 to 0.25µg/mL.
Conclusion: low concentrations of trans-cinnamaldehyde elevate the antimicrobial action of clindamycin, suggesting a possible clinical benefit for utilizing these natural products for combination therapy against *Clostridium difficile*.

Development of SERCA Inhibitors Targeted towards Prostate Cancer Cells

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The ability to produce well ordered crystals of sarcoplasmic reticulum (SERCA) Ca^{2+}-ATPase has resulted in the elucidation of a vast number of the structures of this protein at atomic resolution, corresponding to its various phosphorylated / non-phosphorylated and inhibitor bound states. Thus the specific high-affinity binding site of the sesquiterpene lactone thapsigargin (TG), isolated from the mediterrenan plant *Thapsia garganica*, to the E2 conformations of SERCA 1a has been found in a preformed notch, located at the lipid-protein interface between the M3, M5, and M7 transmembrane segments of the Ca^{2+}-ATPase (Olesen *et al.* 2004, 2007). Although the inhibition of SERCA pumps in both normal and cancer cells precludes the use of TG as an antineoplastic agent , this difficulty can be addressed in the case of prostate cancer by targeting these cells with an inactive prodrug derivative of thapsigargin where the butanoyl group of the guaianolide ring has been replaced by 12-aminododecanoyl conjugated to a BOC- peptide (Søhoel *et al.* 2006). After specific cleavage of the peptide moiety by the PSA (prostate surface antigen) protease, the liberated hydrophobic thapsigargin derivative can cross the plasma membrane to exert apoptosis on the prostate cells (Denmeade *et al.* 2003). Cocrystallization of the TG derivative with rabbit SERCA 1a at 3.3 Å resolution shows that the long flexible hydrocarbon chain of the inhibitor penetrates the transmembrane region of the protein, reaching towards one of the Ca^{2+} binding sites (Søhoel et al. 2006), confirming the potential of this strategy to kill prostate cancer cells. However, due to the strong hydrophobic properties the mentioned prodrug is not ideal for therapeutic use. At the meeting I will report on progress why TG is such a specific and high affinity inhibitor of Ca^{2+}-ATPase, and also on our efforts towards development of a rational approach to synthetize more soluble and equipotent derivatives, suitable as candidates for prodrugs in the treatment of slowly growing and hormone insensitive prostate cancers.
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Inhibition Of Drug Resistance Of Bacteria And Cancer Cells

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Background: Chemotherapy started with Ehrlich's "Magic Bullets" and large number of antibiotics were developed on the basis of his ideas. Resistance to antibacterial and anticancer drugs appeared soon after the introduction of antibiotics and chemotherapeutics, resulting in non-treatable infections and malignancies. This paper reports on the reversal of the multidrug resistance (MDR) of bacteria and tumor cells.
Methods: Plasmid replication, partition and conjugal transfer were inhibited simultaneously in various bacterial species carrying plasmids encoding antibiotic resistance in the presence of phenothiazines and disiloxanes *in vitro*. The inhibition of the ABC transporter of the cancer cells was measured in the presence of phenothiazines and patented disiloxanes by rhodamine accumulation in flow cytometry in human MDR1 gene-transfected mouse lymphoma, human breast and colon cancer cell lines.
Results: Antiplasmid effects of substituted phenothiazines and structurally related compounds were demonstrated on Gram- positive and negative bacteria. Elimination of the antibiotic resistance of R plasmids of *E. coli* and other bacterial species was induced by phenothiazines. The effects of antiplasmid compounds are based upon intercalation into the superhelical form of plasmid DNA and the simultaneous inhibition of conjugal plasmid transfer. The phenothiazine promethazine synergized the antibacterial effect of gentamycin in chronic recurrent pyelonephritis *in vivo*. On the basis of structure-activity correlations, anthril-derivatives with antiplasmid activity were synthetized.
MDR is the main reason for the failure of cancer chemotherapy. The MDR1 activity of human MDR1 gene-transfected mouse lymphoma, human breast and colon cancer cells was inhibited in the presence of resistance modifier phenothiazines and disiloxanes. The disiloxane resistance modifiers synergized the antiproliferative effect of doxorubicin and taxol *in vitro*. In addition the disiloxane reduced the growth rate of cancer cells co-cultivated with normal cells (unpublished results).
Conclusions: The results of model experiments on the synergistic interactions between antibiotics, chemotherapeutics and resistance modifiers on bacteria and cancer cells can be exploited in rational drug design for combination chemotherapy.

Epigenetic Therapy Using 5-Aza-2'-Deoxycytine: A Potential Magic Bullet Against Cancer

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Aberrant DNA methylation, an epigenetic event, plays an important role in tumorigenesis by silencing genes that suppress malignancy, such as tumor suppressor genes (TSGs). Since this epigenetic change is reversible, it is a potential target for 5-aza-2'-deoxycytidine (DAC), a potent inhibitor of DNA methylation that can reactivate TSGs. There are several properties of DAC that make it a potential "magic bullet" to treat cancer: 1) Each tumor has many TSGs that are silenced by DNA methylation. 2) Since DAC is an S phase specific agent, it will only target proliferating cells. 3) The concentrations of DAC that induce a loss of clonogenicity of tumor cells are low (< 1 micromolar). 4) Due to its small molecular size (< 300 Da) and its physicochemical properties, DAC has the great potential to penetrate tumors. One of major reasons for the failure of many cytotoxic drugs to cure cancer is due to their poor penetration into tumors at therapeutic concentrations. 5) The antineoplastic action of DAC can be enhanced by other epigenetic agents, such as inhibitors of histone deacetylase. 6) Drug resistance to DAC can be prevented by the use of agents that inhibit cytidine deaminase, the enzyme that inactivates DAC or agents that are cytotoxic to tumor cells deficient in deoxycytidine kinase, the enzyme that activates the prodrug, DAC. 7) In animal models DAC was shown to be a potent antileukemic agent, more active than cytosine arabinoside (ARA-C) (Momparler et al. Leuk Res 8: 1043, 1984), the major drug for the treatment of acute myeloid leukemia**.** 8) Resting, non-proliferating, hematopoietic stem cells are resistant to DAC and are rapidly recruited into the cell cycle after treatment with this agent to overcome the problem of neutropenia, its major side effect. In preliminary clinical trials on patients with acute leukemia (Rivard et al. Leuk Res 5: 453, 1981) and patients with lung cancer (Momparler et al., Anticancer Drugs 8:358, 1997), DAC showed promising activity. Preclinical studies show that the potent antineoplastic action of DAC is highly dependent on its dose-schedule (Lemaire et al., BMC Cancer 8: 128, 2008). For clinical therapy of cancer the optimal dose-schedule for this interesting epigenetic agent remains to be determined. At the present moment DAC has only been approved for the treatment of the hematological malignancy, myelodysplastic syndrome (MDS). Why does an agent like DAC with so much potential in cancer therapy have only limited clinical use? The major problem is the lack of knowledge on how to translate preclinical results to clinical therapy. This is the same problem that Paul Ehrlich faced in his research on chemotherapy a century ago. (Supported by grants from Canadian Cancer Society & Cancer Research Society).

Ketanserin And Dexfenfluramine: Angels Or Demons For 5-HT_{2B} Receptors?

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Background: Ketanserin and dexfenfluramine are two old drugs that both target the 5-HT_2 Gq-coupled subtypes of serotoninergic receptors. The first was developed as an α_1-adrenergic/5-HT_{2A} antagonist for the treatment of hypertension and withdrawn for the risk of cardiac arrhythmias and sudden death due to QT interval prolongation on the electrocardiogram. This effect is not due to the 5-HT_2 receptor antagonism but to the blockade of HERG potassium channels. The second was employed as an appetite suppressant and is metabolized in norfenfluramine, a powerful 5-$HT_{2B/2C}$R (5-$HT_{2B/2C}$R) agonist. Its agonist property at 5-HT_{2B}R explains the main adverse effects of the drug in heart and lung (i.e. pulmonary hypertension and cardiac valves fibrosis). In the presentation, we will show the main cardioprotective effects associated with 5-HT_{2B}R blockade.
Methods: Standard cardiovascular phenotyping methods were applied to 5-$HT_{2B}R^{-/-}$ mice and animals overexpressing the 5-HT_{2B}R in cardiomyocytes. Then wild-type treated with 5-HT_{2B}R antagonists and 5-$HT_{2B}R^{-/-}$ mice were submitted to chronic infusions with a β-adrenergic agonist (isoproterenol) or angiotensin II. Cardiac function and anatomy were measured by echocardiography together with the measurement of blood pressure parameters. We also measured plasma inflammatory cytokines and oxidative stress in heart and vessels. Finally, the cellular-coupling of the 5-HT_{2B}R was analyzed in left-ventricular fibroblasts.
Results: The 5-HT_{2B}R is crucial in adult cardiac hypertrophy due to pathological stimuli such as isoproterenol and angiotensin II. Its blockades reduces cardiac production of inflammatory cytokines and myocardial oxidative stress. Most of these actions are taking their origin in ventricular fibroblasts.
Conclusions: For cardiac remodelling, the 5-HT_{2B}R appears as a magic bullet. Nevertheless, the "power of the past" has yet limited the clinical development for selective 5-HT_{2B}R antagonists.

First-Line Treatment With Rituximab Combined With Intravenous Or Oral Fludarabine For Patients With Extranodal Mucosa Associated Lymphoid Tissue (MALT) Lymphoma

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Background: The addition of Rituximab has improved the outcome B-cell NHL and Fludarabine, alone or in combination, has been effective in MALT lymphoma. Also, our *ex vivo* preliminary results indicate a synergistic antitumor effect on MALT cells with combination of Fludarabine and Rituximab. This work evaluates the safety and efficacy of the combination of Fludarabine and Rituximab in the first-line treatment of extranodal MALT lymphoma.
Methods: This study enrolled 22 adult patients with extranodal MALT lymphoma, who had not received previous chemotherapy and who require systemic treatment. Patients received Rituximab 375 mg/m^2 intravenously (IV) on day 1 and Fludarabine 25 mg/m^2 (IV) on days 1-5 (days 1-3 when > 60 years), every 4 weeks; after the first cycle, oral Fludarabine was allowed to be given orally at 40 mg/m^2 with the same schedule. An evaluation was done after three cycles and patients in complete emission (CR) received an additional cycle and those achieving only partial response (PR), a total of 6 cycles.
Results: 18 included patients have been already started on therapy: median age: 59 years (range: 32-83); 7 male, 11 female; PS 0 (94%); site of lymphoma origin: stomach (61%), skin (16%), lung (11%), parotid gland (11%); stage: I (66%), II (16%) and IV (16%). 2 pts. received 2 cycles, 9 pts. 4 cycles, 7 pts. 6 cycles. 17 pts. were evaluable for response. Overall response rate was 100% with 94% achieving CR and another one PR. One patient with initial parotid gland involvement relapsed after 6 months in two previously unaffected areas (breast and bone marrow). Median follow-up was15 months (range: 17-27). PFS rate is 93% (CI95%: 79-100%) at 12 m and OS rate 100% at 12 m. Tolerance to oral Fludarabine was excellent. Mild neutropenia was the most common toxicity, usually occurring after the third cycle.
Conclusions: These preliminary data indicate that the RF regimen, both with intravenous or oral Fludarabine, was well tolerated and is very active in the first line treatment of extranodal MALT lymphoma, even with fewer cycles than initially planned.

Synthesis, In Silico ADME-Tox Study And Antimicrobial Evaluation Of Some Small Molecules

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Background: The development of antimicrobial agents from 1,2,4 triazole (TZ), 1,3 dihydrobenzimidazole-2-one (DBO) and 1,3 dihydrobenzimidazole-2-thione (DBS) derivatives were attempted to synthesized and evaluated for antifungal and bacterial activity. In silico pharmacokinetic study (PK) on the synthesized compounds were performed for its drug likeliness.
Methods: A novel Schiff bases of TZ derivatives was prepared from thiocarbohydrazide and 3,5,-dimethoxy benzoic acid followed by treating with corresponding substituted benzaldehydes. DBO and DBS derivatives were prepared by microwave method. The structures of all synthesized compounds were conformed by analytical methods. Antimicrobial activity was finding out in gram positive, gram negative and fungal strains. In silico PK study was done on Pallas software.
Results: Antimicrobial activity of the DBO compounds showed that aromatic substitutions have considerable activity against S. aureus and C. albicans.
The 2-substituted DBS derivatives posses better activity against all the strains (S. aureus, S. epidermitis, B. subtilis, E. coli, K. pneumoniae, P. vulgaries, C. albicans) in 50 mg/ml. All the compounds have significant activity against C. albicans.
In silico PK prediction shows that all the compounds obeyed Lipinski rule of 5 and have free of toxicity and metabolically stable.
Conclusion: From the study, it was concluded that Bulky substitutions in the 2nd position of DBO increase the activity than parent compound.
In DBS, 2 substituted compounds have significant activity against the microbial strains than substitution in 1st position. Except napthylamine, other compounds have negligible activity against E. coli and maximum activity with B. subtilis.
Chloro, dichloro and hydroxyl derivatives of TZ have significant activity at 64 mg/ml against C. albicans.
In silico PK study shows that the synthesized compounds except oxiranyl and napthylamine derivatives of DBO and Amino, methyl derivatives of TZ have probability of toxicity and others are free from toxicity.
From the study it is concluded that the bulky ring is necessary for the activity while the ring should not undergo any epoxide metabolite formation.

One Bullet, Two Targets! A Nano-Particle With Two Key Medical Applications

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Background: How can an efficient low-to-no toxic adjuvant nano-particle concept rest on the same concept as a cancer cell killing drug? The 40 nm ISCOM nano-particles are based on acyl-saponin (ASAP) and desacyl-saponin (DSAP) from *Quillaja* saponin (QS). They exert their activities by guiding cells of lymphoid origin to activation measured by down regulation of CD14 on immature dendritic cells (DCs) and expression of CD83 to differentiation measured by production of proinflammatory cytokines and expression of communication molecules e.g. CD86. That is a normal pathway leading to the programmed cell death i.e. apoptosis virtually without any side effects. More than 450 publications have demonstrated that ISCOM formulations are potent adjuvants with strong immune modulating capacity. ISCOMs are now in human phase 3 studies. To note the free i.e. non-particulate QS and the ASAP fraction are highly cell lytic due to interaction between the saponin and the cholesterol in the cell membrane. By saturating the acyl-saponin with cholesterol non-lytic particles can be formed like ISCOMs that are well tolerated by man and animal.
Methods-Concepts: The present immune adjuvant and cancer cell killing concept is developed from the ISCOM technology used as adjuvant formulations. Two 40 nm particles i.e. KGI containing ASAP and BBE containing DSAP were formulated using the same technology. A blocking technique was applied to analyse interaction of QS nano- particles with cancer cells revealing two receptor systems. Various methods were used to demonstrate lytic, necrotic and to apoptotic cell death as well as the exit of the cancer cells from the cell cycle. Various cancer cells including leukemic cells and solid tumour cells were exposed to the KGI particles and the cell survival was measured by the Alamar Blue and also other methods.
Results: The nano-particles KGI and BBE mediate their effects by communicating with the target cells by a primary attachment receptor common to ASAP and DSAP having a functional modulating capacity and a second receptor unique for ASAP most likely located on the acyl-chain. ASAP in the KGI has an additional strong modulating device for apoptosis different from the device of the common receptor. KGI particles killed cancer cells at 30 to 40 fold lower concentrations than normal cells by apoptosis after taking the cancer cells out of the cell cycle, causing activation differentiation including cytokine production. The immune modulatory and cancer cell killing effects of ASAP (KGI) and DSAP (BBE) particles differ resulting in synergistic adjuvant and cancer killing effects and also they potentiate the effect of standard anticancer drugs measured *in vitro*. The KGI and BBE particles have shown cancer cell killing effect on cells from about 10 different lines including cells from solid tumours.
Conclusion: QS particles can be used as vaccine adjuvant and as anti-cancer drug following the same biological mechanisms including activation, differentiation and eventually apoptosis. 1) The dual KGI - BBE system is a new low-to non-toxic concept applicable for immune adjuvants, which has already been well established, as well as for efficient induction of apoptosis of cancer cells with a high therapeutic index, which is a new discovery. 2) The dual particle system has been shown *in vitro* to kill a variety of cancer cells including cells from solid tumours. 3) The particles can also act synergistically with other standard anti-cancer drugs. In short, the dual ASAP and DSAP particle concept is an efficient, low-toxicity, high bioavailability vaccine adjuvant and cancer killing system based on initiating normal cell differentiation. The stand alone and synergistic effects between the components of the system, provides great promise for strong developments in two medical fields.

A Unique SPE-LC-MS/MS Platform For Fully Automated Analysis Of Drugs In Native Whole Blood

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Background: Current protocols for the pretreatment of whole blood samples upfront to LC-MS/MS analysis of drugs or endogenous compounds involve manually performed multi-step operations such as protein precipitation and centrifugation or drying/elution of blood spots on filter paper. All these clean-up methods hardly can be automated or integrated into an on-line analysis procedure. In order to achieve this goal we developed a unique procedure which converts anticoagulated whole blood into a novel matrix which can be further processed on-line by different sample clean-up methods, such as Solid Phase Extraction (SPE).
Methods: For the transformation of whole blood into so called cell-disintegrated blood (CDB) we apply a heat-shock treatment. After mixing and injection of 20 µL of anticoagulated blood by an appropriate autosampler system (Symbiosis Pharma, Spark Holland) the sample is pumped at a defined flow-rate through a heated stainless-steel capillary (300 x 0.5 mm ID) set at 75°C. The resulting CDB then is flushed on-line onto a SPE-cartridge (20 x 1 mm ID, packed with Oasis HLB) and further processed. The extracted analyte fraction (immunosuppressants) finally is eluted onto a LC-column (LiChrospher 100 RP 18 EC, 125 x 2 mm ID), separated and detected by a tandem mass spectrometer (Quattro Micro, Waters) operated in ESI (+) MRM mode.
Results: The described in-line processing, i.e. the conversion of an anticoagulated whole blood sample into CDB yields a homogeneous, red-coloured biofluid composed of subcellular particles (≤ 1 µm) which do not sediment. The heat-shock quantitatively disintegrates erythrocytes, leucocytes and up to 60% of thrombocytes. A comparison of drug levels in whole blood samples of immunosuppressed patients measured by the described fully automated method and those obtained with the established manually performed precipitation ($ZnSO_4$/MeOH) procedure revealed a very good correlation.
Conclusions: We developed a fully automated method by which an anticoagulated whole blood sample is processed in such a way that the generated cell-disintegrated blood (CDB) fluid can be further subjected on-line to conventional bioanalytical extraction, fractionation and / or separation methods (e.g. SPE, LC and CE) as well as detection modes.
Supported by EUREKA, Project E!4112

Pharmacokinetics (PK)/Pharmacodynamics (PD) Of Infliximab In Treatment For Patients With Rheumatoid Arthritis: Characterization Of Infliximab-Resistant Cases And PK-Based Modified Therapy

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Background: Infliximab, a chimeric anti-tumor necrosis factor α (TNFα) monoclonal antibody, has provided a significant impact on management of rheumatoid arthritis (RA); however, a subset of patients shows poor therapeutic responses. The purpose of this study is to understand the mechanism underlying such unresponsiveness, by considering clinical PK/PD properties of infliximab and to present modified therapy for such poor responders.
Methods: Twenty-one patients with active RA were scheduled to receive an intravenous infusion of infliximab (3 mg/kg or 200 mg) at weeks 0, 2, and 6, followed by maintenance therapy every 8 weeks. We examined a relationship between clinical responses and trough serum concentrations of infliximab at week 14. We also measured time-serum concentration profiles and values of PK properties in individual patients for each infusion of infliximab and compared them with disease activities in their clinical courses.
Results: Fifteen cases achieved good or moderate responses in the European League Against Rheumatism criteria, and 3 cases resulted in nonresponders at week 14. The means of distribution volume and elimination half-life ($t_{1/2}$) during the first 2 weeks were 0.05 L/kg and 9.5 days, respectively. Through 14 weeks, most good and moderate responders maintained trough serum concentrations of more than 1 µg/ml. Only 3 cases showed undetectable levels of trough serum concentration at week 14. By contrast, the PK profiles of all nonresponders except one showed rapid clearance during therapy. We also found that the $t_{1/2}$ during the first 2 weeks is inversely correlated to the disease activity scores at the start of therapy. For patients with a rapid clearance of infliximab, the increased use of prednisone or methotrexate was a useful way to achieve sufficient clinical responses. The addition of tacrolimus was effective to improve the clinical outcomes of nonresponders.
Conclusions: (1) Maintaining the trough serum concentrations of infliximab above therapeutic limit levels is beneficial for favorable clinical outcomes. (2) The rapid clearance appears to be the main course of unresponsiveness of infliximab. (3) PK data apparently offer guidance when an optimal treatment for infliximab-resistant RA patients is being considered.

Cytotoxic Diterpenes From Australian Flacourtiaceae As A Good Source Of Antineoplastic Agents

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Background: Search for new anticancer agents (e.g. taxol) from Plant is still an important field to Phytochemist and other related scientist. Flacourtiaceae is an under exploied family and no chemical and biological works have previously been reported on any Australian Flacourtiaceae. Therefore the main objective of our study was 1) To evaluate the cancer cell line based (*in vitro*) cytotoxic effects of diterterpens isolated from Australian Flacourtiacae 2) To provide a useful lead compound for future antineoplastic drug development 3) To gather more data to contribute to a better understanding of the Flacourtiaceae.
Methods: In this study, total threeteen novel diterpenes had been isolated from three Casearia genera of Australian Flacourtiaceae.Two rare dialdehyde clerodane diterpenes [1] and [2] and one unusual mixed acetoxy and methoxy acetal clerodane diterpenens had been isolated from root of *Casearia multinervosa* by column chromatography followed by semi-preparative HPLC using an RP C_{18} column. Similarly, leaves and stems of *C.grayi* and *C. grewiifolia* yielded seven and five clerodane diterpenes respectively, among them two were common in both species and an excepmtional optical active unsaturated keto-diacetal [3] clerodane diterpenes was obtained from Ethyl acetate extract of narrow stem of *C. grayi*, by preparative HPLC employing variations on a gradient elution binary system of acetonitrile and water. All the isolated diterpenes were characterized with means of various spectroscopic methods (e.g. UV, IR, NMR, LC-MS etc). All the diterpenes were tested for cytotoxicity against various cancer cell line using ATP Lite-M assay method with a reference compound chlorambucil. All the cells were obtained from American Type Culture Collection (ATCC) and were routinely cultered with essential medium. Each sample was tested in triplicate and resultes were expressed as LC_{50} values (µM).
Results: All the diterpenes were tested for cytotoxicity against P388 cell line and all showed cell inhibition in excess of 92% at a concertration of 0.02 mg mL^{-1}. The most post potent dials [**1** and **2**] and keto-acetal [**3**] diterpenes, were futher examined against five cancer cell line (A375, HepG2, Hs27, P388 and PC3) and exhibited significant cytotoxicity compared to that of the reference compound chlorambucil (see the following Table).

Compound	Cell line				
	A375	HepG2	Hs27	P388	PC3
1	2.1	2.0	1.9	2.0	2.1
2	1.5	1.6	1.5	2.0	2.0
3	2.0	2.0	1.5	1.9	1.8
Chlorambucil	70.1	27.4	93.0	24.5	39.7

Conclusions: In the investigantion, all the isolated compounds were the first example of clerodane diterpenes isolated from a plant source. Allthough the cytotoxic activities of three novel clerodane diterpenes [**1-3**] were appreciable, none of them showed selectivity for any cell line, indicating that the compounds were functioning as a gerneral cytotoxic agents. This finding suggest that the cytotoxic diterpenes from Casearia genera of Australian Flacourtiaceae would be a good source of antineoplastice agents. Therefore, more extensive work should be carry out to isolated bioactive compounds from Australian Flacourtiaceae and to understand their machanism of action.

The Unexpected Hidden Face Of The Cephalosporin Antibiotic Ceftazidime: From Biological To Chemical And Physical Activities Against Oxidant Species Produced By Phagocytes

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Background: Over several decades the fight against gram negative bacteria infections is a main challenge, and ceftazidime (CAZ), a cephalosporin's family compound, is largely used in intensive care units to treat severe sepsis. Here in, we demonstrated that, beside its antibiotic effect, CAZ has unexpected antioxidant properties, and tried to better understand its mechanism of action.
Methods: Five *in vitro* (cell-free systems) and *ex-vivo* experimental models were designed to assess the capability of CAZ to protect 1) the trypsin (1.4 µg) inhibitory activity of alpha$_2$-macroglobulin (□$_2$M) submitted to an oxidant inactivation by stimulated phagocytes (PMNs) 2) human endothelial cells in culture against the toxicity induced by myeloperoxidase (MPO) or stimulated PMNs 3) alveolar cells (A549, 10^{10} cells/ml; adherent or in suspension) against the oxidant damage induced by anoxia/reoxygenation, using oxymetry and EPR-spin trapping techniques 4) linoleate from lipoperoxidation induced by □-irradiation, Fe^{2+}/ascorbate system or ferryl species; 5) against hydroxyl radical or singlet oxygen ($^1O_2$) produced respectively by the Fenton (Fe^{2+}/H_2O_2) and the Mallet (H_2O_2/NaOCl) reactions. All experiments were done at least 5 times.
Results: CAZ at 10^{-3}M had a significant protective effect on □$_2$M against oxidative stress; CAZ had a significant dose-dependent (from 10^{-5} to 10^{-3}M) inhibitory capacity against MPO toxicity on endothelial cells and protected A549 cells from an excessive production of free radicals during anoxia/reoxygenation. In cell-free systems, CAZ at 10^{-4} M significantly protected linoleate from lipoperoxidation and had a unique $^1O_2$–deactivating activity by scavenging $^1O_2$ energy. CAZ was less active on ferryl species, acting by a chelating activity on iron.
Conclusions: Overall results indicate that: 1) CAZ, beside its antibiotic activity, has a protective activity against oxidant species 2) due to these unexpected properties, the clinical use of CAZ, which is essential to fight against bacteria, may have beneficial side effects in inflammatory and anoxia/reoxygenation situations where excessive activation of leukocytes and increased production of ROS are described.

Pharmacogenetic and pharmacokinetic approaches after kidney and liver transplantation

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Immunosuppressive drugs such as cyclosporine (CsA), tacrolimus (Tac) and sirolimus (Sir) are characterized by a narrow therapeutic index and display wide interindividual variability in the oral dose, to which many factors contribute. Special interest in polymorphisms in genes encoding biotransformation enzymes and drug transporters has offered new insights towards a better understanding of this variability. Several single nucleotide polymorphisms (SNPs) have been identified in both *CYP3A4* and *CYP3A5*. A particular SNP within intron 3 of the *CYP3A5* gene results in expression of the active CYP3A5 enzyme when associated with the wild-type *CYP3A5*1* allele, and in production of a truncated protein when associated with the *CYP3A5*3* allele. *CYP3A5* SNP has been shown to influence the pharmacokinetics of calcineurin inhibitors (CsA, Tac) and Sir after solid organ transplantation. Other SNPs have also been identified in the *ABCB1* gene encoding P-glycoprotein (P-gp), and those in exons 11, 12, 21 and 26 have been extensively studied in organ transplantation. However, the association between calcineurin inhibitor (CNI) pharmacokinetic parameters and the *ABCB1* genotype/haplotype is still a matter of debate.
We observed that post-transplantation renal function could be affected in the long term by genetic polymorphisms of the donor in patients treated with CNIs, which has enhanced our understanding of factors affecting long-term graft survival. After liver transplantation, Tac concentrations in hepatic tissue showed a significantly better correlation with the severity of organ rejection than pre-dose blood levels. In addition, *ABCB1* polymorphisms were found to significantly influence Tac hepatic concentrations.
In conclusion, *CYP3A5* polymorphism is undoubtedly closely associated with Tac disposition. P-gp activity in the renal parenchyma appears to be a relevant factor linked to CNI nephrotoxicity, and *ABCB1* genotyping of donors and recipients may serve as a marker for CNI toxicity risk assessment. However, a causal relationship between *ABCB1* SNPs, P-gp activity, intrarenal CNI concentrations and drug-related nephrotoxicity still needs to be confirmed by further studies. The contribution of a genetic approach to pharmacokinetic modeling programs may thus optimize dosing strategies after organ transplantation.

Role Of Ultrasound Waves In Enhancing The Effect Of Antitumor Drug

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Background: Chemical activation of drugs by ultrasound energy for treatment of cancers is a new field recently termed "sonodynamic therapy". The combination of a drug and ultrasound has a killing effect on the cell . Despite this fact very little is known about ultrastructural changes of tumor cells after treatment with anticancer drug in combination with ultrasonocation. Also, the mechanism of sonodynamic action in tumor cells is poorly investigated. Aims: 1) To evaluate the role of ultrasonic waves in enhancing the effect of 5-fluorouracil (5-FU) as anticancer drug. 2) To study the physical parameters, which enhance ultrasonic effect on anticancer drug transportation through biological membrane.. 3) To propose optimized dosage regimens.
Methods: To evaluate the role of ultrasonic waves in enhancing the effect of 5-fluorouracil (5-FU) as anticancer drug, tumor growth, cell ultrastructure and temperature rises of ascites Ehrlich tumor implanted in mice were investigated at a frequency of 0.8 MHz. Different conditions of ultrasonic intensity (1, 2 and 3 W/cm^2), sonocation time (1, 3 and 5 min) and waveform (continuous and pulsed) were studied. A total of 10 mg/kg body weight of 5-FU was IV injected into the mice bearing Ehrlich tumors on days of 1, 3, 5, 8, 10 and 12 of therapy. After 24 h of each injection with 5-FU, tumor sites were sonocated at room temperature of 22°C. Tumor volumes were monitored by using ultrasonic imaging system during treatment just before each 5-FU injection, and on the 15th day of treatment. Density and ultrasonic attenuation of excised tumor tissues were measured *in vitro* and used to estimate the temperature rises due to ultrasonic absorption.
Results: Tumor volumes were monitored by using ultrasonic imaging system during treatment just before each 5-FU injection, and on the 15th day of treatment. Density and ultrasonic attenuation of excised tumor tissues were measured *in vitro* and used to estimate the temperature rises due to ultrasonic absorption. Density and attenuation coefficients of excised tumor tissues were found to be dependent on the treatment regimen. The estimated rate of temperature rise and equilibrium temperature, and the characteristic time to reach equilibrium are given for each group. Results obtained indicate that tumor growth decreases with increasing of ultrasonic intensity and sonocation time. Tumor growth was delayed 4 to 6 days by combined treatment of 5- fluorouracil and ultrasound (US). Ultrastructure investigations of tumor cells showed severe damage in cytoplasmic organelles and cytoplasmic vacuoles that increased with increasing ultrasonic intensity and sonocation time. This damage appears as prominent crowded vacuoles among swollen ruptured organelles, chromatin fragments and severe increase in numbers of pyknotic and apoptotic cells.
Conclusions: The combination of 5-FU and ultrasound produced significantly greater antitumor activity than ultrasound or 5-FU alone.

Investigations Into Sulfur Nanoparticles As Drug Carriers

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Background: Inorganic nanoparticles are used in the field of in vivo diagnostic nuclear medicine practice. E.g. ^{99m}Tc labeled sulfur colloid dispersions are used in sentinel node detection. After injection the nanoparticles are taken up by the lymphatic system. This uptake mechanism could also be used in the field of drug targeting. It is the aim of our studies to perform basic investigations into the potential of inorganic colloidal particles as drug carriers.
Methods: Sulfur colloids were prepared by redispersion of colloidal sulfur (method M1) or by synproportionation of sodium sulfide and sodium sulfite in a solution containing sulfuric acid (M2). The formed particles were used as reference. Caffeine was added during the particle formation process (M2). PCS (photon correlation spectroscopy) measurements were performed (Malvern Autosizer IIc) after filtration (1.2 resp. 0.45 µm cellulose nitrate). SEM (scanning electron microscopy) (Cam Scan, Electron Optics LTD) was used to visualize the nanoparticles, which had been collected on 0.1 µm filters (polyether sulfonate). The caffeine concentration was determined UV spectroscopically (Specord S100, Analytik Jena).
Results: Sulfur nanoparticles in colloidal range were formed. The size distribution was not small according to PCS results. The particles were stable enough to be collected on filters and examined using SEM. The formation of the sulfur nanoparticles was influenced by the presence of the model drug caffeine. Particles with a mean diameter of 73 ± 6 nm were measured (PCS, n=6). The polydispersity index is smaller compared to the value calculated for drug-free sulfur nanoparticles. The nanoparticle dispersions containing caffeine were further investigated (n=6). By using filters (0.45µm and 0.1 µm), fractions of nanoparticles were separated, the remaining particle dispersions were investigated. About 4.7 % (w/w) of the total caffeine amount was assigned to the colloidal fraction. To get information if caffeine interacts with the surface of the sulfur particles, caffeine was added to colloidal sulfur. Even after 2 hours, the absorption of drug was negligible.
Conclusions: The presence of the model drug caffeine has an influence on the particle formation of sulfur nanoparticles, which are manufactured by synproportionation. Surface adsorption as mechanism of interaction between caffeine and sulfur nanoparticles is not relevant.

Teaching An Old Dog New Tricks: Intralesional Bleomycin Injection (IBI) Treatment Of Vascular Birthmarks

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Background: We have provided a novel use to an old chemotherapeutic agent as a scar less injection treatment of vascular birthmarks for the last three years. 82 of 300 patients seen in our centre received intralesional Bleomycin injection treatment.
Methods: Clinical response, administered dose, amount of sessions and complications is recorded. The following pathologies were treated thus far: Hemangioma 18 pts, venous malformation 49, cystic hygroma 2, lymphatic malformation 6, Arterio-venous malformation 2, and mixed malformations 5.
52 of the 82 patients completed their treatment. Respiratory surveillance is provided by an adult and paediatric pulomonologist utilising the locally agreed Cleveland malformation surveillance protocol.
Results: 19 children and 36 adults completed treatment with a mean of 4.2 injection sessions. Treatment lasted for an average of 106 days. Complete resolution occurred in 56%, with an overall response rate of 96%. Skin ulceration occurred in 1 patient, blistering in 5, infection 1, swelling 1, headache 1, bruising 1, rash 1 and skin pigmentation occurred in 3 patients. One patient presented with a major complication needing ITU admission and ventilation following treatment of a pan-facial and thoracic lymphatic malformation. The maximum administered dose was 3mg/kg.
Conclusion: As a single site under the auspices of a multi-disciplinary team, the Cleveland Vascular Malformation Group, we have successfully treated complex and recurrent vascular anomalies. The data and outcome results provide a reference and experience of a national single site in treatment of these challenging lesions.

Indocyanine Green: Revisiting An Old Friend

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Background: Indocyanine green clearance, measured by percentage disappearance rate, detects alterations in liver function and may be used as a non-invasive determinant of hepatic reserve. The aims of this study were to compare liver histology and Child's-Pugh score with percentage disappearance rate (PDR) and determine which variables correlated with PDR.
Methods: Child's-Pugh score, liver function tests, liver biopsies and indocyanine green testing (0.5mg/kg) were performed in 102 consecutive patients with cirrhosis of diverse etiologies. Indocyanine green concentration was determined using spectrophotometric analysis (806nm) and plotted logarithmically with Michaelis-Menten kinetics to calculate the PDR. Liver biopsies were graded using the modified Knodell score to obtain a histological activity index.
Results: In multivariable analysis, percentage disappearance rate significantly correlated with Child's-Pugh score, albumin, bilirubin, prothrombin time and histological activity index. Albumin, prothrombin time and histological activity index were independent predictors of percentage disappearance rate in the final model (albumin $p<0.01$, prothrombin time $p<0.046$, histological activity index $p=0.033$), accounting for 46.2% of variability in percentage disappearance rate measurements.
Conclusion: Percentage disappearance rates correlated with Child's-Pugh scores in this series of cirrhotic patients. However, 46.2% of its variability was accounted for by albumin, prothrombin time and histological activity index.

A Novel Biomimetical Transformation To Construct Natural Product Frameworks

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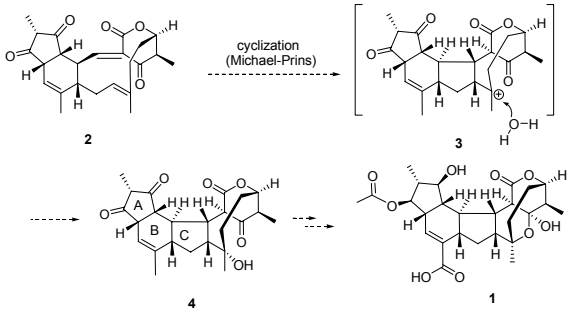
The chemical synthesis of natural product and their derivates has become an integrated part of developing pharmaceutical drugs. Its role is especially important in producing a scarce, but biologically intriguing, natural product in larger quantities for further biological investigations and medicinal applications. [1] During the last century, the synthetic approach has been strived for to apply the biogenesis consideration, aiming at the simulation of biogenetic key step which mimic nature in its elegance and efficiency. [2] Such challenge has motivated our group to involve a biomimetic transformation in our synthetic plan of a potent anti-tumor agent hexacyclinic acid 1. [3,4,5]
Central to the strategy is the biomimetic Michael-Prins reaction to construct the C ring and to deliver the tertiary alcohol, resulting from water addition to carbenium ion intermediate.



Scheme 1. Biomimetic route utilizing Michael-Prins reaction in hexacyclinic acid **1** synthesis
Through our investigation, we were able to conduct the above reaction which provides not only a new C-C coupling reaction but also a Lewis acid-catalyzed reaction that could be carried out in aqueous media under a mild condition with a high level of catalyst recovery. This could give chance for pharmaceutical companies to synthesize efficiently and deliver more environmentally friendly reactions.
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Cell Penetrating Antibody Delivery To Intracellular Targets

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Background: A limitation of developing therapeutic antibodies specific for intracellular structures is to allow antibodies to enter cells without loosing cell viability. The objective of this project was to create antibodies for intra-cellular targets to control cell proliferation.
Methods:. Trans-membrane antibodies were generated by photo-affinity cross-linking a 17mer peptide derived from human sarkosi sarcoma virus. Targeting of antigens inside live cells is demonstrated by confocal microsopy, and functionally by inhibition of apoptosis.
Results: Trans-membrane penetrating (TMP) antibodies stained in live cells specifically actin and paxillin while naked antibodies did not. TMP antibodies also did not affect cell growth in culture. Furthermore, TMP modified anti-caspase-3 antibodies were shown to penetrate human T cell lymphoma cells, and rapidly inhibit actinomycin D-induced apoptosis.
Conclusions: Membrane penetrating peptide-modified antibodies are endowed with a potency of targeting intra-cellular antigens in living cells and tissues, suggesting the clinical potential of immuno-therapeutic TMP-antibody delivery by cell-penetration.

Diagnosis And Innovative Therapy Of Human Specific *Enterocytozoon Bieneusi* Genotype D Strain In Falcons (*Falconiformes*)

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Background: Out of 137 falcons of the same group, 24 falcons died from clinically unidentified abscesses in intestines, liver and kidneys within 6 weeks. 70 more falcons suffered from the same disease. Aims: 1) To identify the causative agent. 2) To establish an appropriate treatment regime and to reduce mortality rate of the sick falcons.
Methods: This study included 137 falcons of 5 breeds. Blood hematology and biochemistry, parasitology, radiography and endoscopy were performed in all birds as well as virological and serological tests. Necropsy, histopathology and immunohistochemistry of the dead falcons was performed. 6 liver and intestine samples were tested by universal diagnostic PCR for microsporidia followed by a second, species specific, PCR for confirmation of *E. bieneusi.* Gyr-Hybrid falcons revealed a higher morbidity rate of 59.2%. Intestinal abscesses were found in up to 50% of the sick birds. All falcons were treated with Dimetronidazole (Emtryl®) 50 mg/kg SID po for 10 days with 3-4 repetitions up to maximum 40 days. Individual treatment plans included the application of Nux vomica® 1ml/kg SID po and Mucosa compositum® 1ml/kg SID po in case of intestinal abscesses, Hepar compositum® 1ml/kg SID po and Legalon® 1 tab BID for 5 days for treatment of liver abscesses, and in case of kidney abscesses Berberis compositum® 1ml/kg SID po and Cantharis compositum® 1ml/kg SID po. Treatment progress was monitored through repeated endoscopies in 2-weeks intervals.
Results: After 5 months, the PCR tests revealed as causative agent the presence of *Enterocytozoon bieneusi* belonging to genotype D identical to the AF101200 strain (human). In total, 24 falcons died shortly after the initial examinations due to advanced disease stages. All other falcons responded well to the individual treatment plan. Repeated endoscopic examinations showed the continuous regression of the abscesses up to their full disappearance and complete recovery of the patients.
Conclusions: 1) Microsporidiosis caused by *Enterocytozoon bieneusi* as new parasitic disease in falcons. 2) Identification of *Enterocytozoon bieneusi* Genotype D Strain for the first time in raptors and falcons might indicate its possible zoonotic potential. 2) Individual treatment plans with Dimetronidazole supported by homeopathic medicines led to a full regression and survival of the diseased falcons.

Alveolar Echinococcosis: The Impact Of Chemotherapy And Surgery On Survival Authors

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Background/Aims: Alveolar echinococcosis (AE) is a serious liver disease. We have recently analyzed the long term prognosis of AE-patients, the burden of this disease in Switzerland and the cost effectiveness of treatment.
Methods: Relative survival analysis was undertaken using a national database with 329 patient records. 155 representative cases had sufficient details regarding treatment costs and patient outcome to estimate the financial implications and treatment costs of AE.
Results: For an average 54-year-old patients diagnosed with AE in 1970 the life expectancy was estimated to be reduced by 18.2 and 21.3 years for men and women, respectively. By 2005 this was reduced to approximately 3.5 and 2.6 years, respectively. Patients undergoing radical surgery had a better outcome, whereas older patients had a poorer prognosis than younger patients. Costs amount to approximately ?108,762 per patient. Assuming the improved life expectancy of AE patients is due to modern treatment the cost per DALY saved is approximately ? 6032.
Conclusions: Current treatments have substantially improved the prognosis of AE patients compared to the 1970s. The cost per DALY saved is low compared to the average national annual income. Hence, AE treatment is highly cost effective in Switzerland.

Calcium Signaling And Angiogenesis

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Background: The proliferation and motility of vascular endothelial cells (EC) are critical steps in angiogenesis and are strictly controlled by different extracellular signals. Proangiogenic factors (VEGF, bFGF) generate cytosolic calcium rises through calcium entry from extracellular medium: this event is due to the opening of calcium-permable channels in the plasmamembrane, mainly activated by arachidonic acid (AA) and nitric oxide (NO). Even if some calcium entry blockers are under clinical trial at present with encouraging results, a better knowledge about the molecular nature of calcium channels and their intracellular regulation could lead to new and more specific strategies in therapeutical approach to cancer progression and angiogenesis.
Methods: Single cell calcium measurements by fluorescent probes, patch clamp, tubulogenesis assay, wound healing, time lapse, PCR, siRNA, proteomics.
Results: Here we describe proangiogenic intracellular calcium signals in endothelial cells derived from human breast carcinoma (B-TEC). AA, released upon EC stimulation with VEGF or FGF, promotes B-TEC proliferation, migration and organization of vessel-like structures *in vitro*. AA induces Ca^{2+} entry in the entire capillary–like structure during the early phases of tubulogenesis *in vitro:* no such responses are detectable in B-TECs organized in more structured tubules. An inhibitor of Ca^{2+} entry and angiogenesis, Carboxyamidotriazole (CAI), significantly and specifically decreases AA-induced B-TECs tubulogenesis, as well as AA-induced Ca^{2+} signals in B-TECs. Finally, preliminary results suggest that at least part of AA-dependent calcium entry could be due to the opening of TRPV4, a well known calcium-permeable channel.
Conclusions: 1) AA-activated Ca^{2+} entry is associated with the progression through the early phases of angiogenesis, mainly involving proliferation, motility and tubulogenesis, and it is downregulated during the reorganization of tumor-derived endothelial cells in capillary-like structures, 2) inhibition of AA-induced Ca^{2+} entry may contribute to the antiangiogenic action of CAI.

The Antitumor Action Of Neurokinin-1 Receptor Antagonists As New "Magic Bullets" Called "Intelligent Bullets"

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Background: In the 21st century, the era of "molecularly targeted" anticancer therapy, the Paul Ehrlich's concept of the "Magic Bullets" for cancer cells, can be useful for neurokinin-1(NK-1) receptor antagonists. Substance P (SP) is a neuropeptide belong to tachykinin family, SP after binding to the NK-1 receptor regulates many biological functions and it is implicated in behaviour emotional, inflammation, pain, mitogenesis, angiogenesis and migration of tumour cells. NK-1 receptor antagonists (L-733,060/L-732,138) were used in the treatment of inflammatory diseases; produce analgesia, antidepressive, anxiolytic and antiemetic effects *in vivo* studies. We have carried out *in vitro* studies of the growth inhibition capacity of these NK-1 receptor antagonists against glioma, neuroblastoma, melanoma, retinoblastoma, pancreas, larynx and gastrointestinal carcinoma cell lines.
Methods: Coulter counter was used to determine viable cell numbers followed by application of the tetrazolium compound MTS. An immunoblot analysis was used to determine the NK-1 receptors, and the DAPI staining method was applied to demonstrate apoptosis.
Results: We have demonstrated the presence of several NK-1 receptor isoforms in all human tumor cells studies. Nanomolar concentrations of SP increased the growth of all cell lines and micromolar concentrations of L-733,060/L-732,138 inhibited the growth of such cell lines in a dose-dependent manner, with and without previous administration of SP. After administration of L-733,060/L-732,138 apoptosis was observed in all tumor cell lines studies.
Conclusions: We demonstrated that: 1) The NK-1receptors were expressed in all these tumor cell lines, 2) SP is a potent mitogen in these tumor cell line, 3) the antitumor action of the NK-1 receptor antagonists L-733,060/ L-732,138 against these human tumor cell lines occurs through the NK-1 receptor and 4) the cell death is apoptosis pathway. This new findings suggests that the NK-1 receptor antagonists are a new and promising antineoplastic agents, called "Intelligent Bullets" concept goes beyond that "Magic Bullets" are to attack the tumour cells invaders antitumor action, but in addition, in the host they present/display beneficial effects such as: anti-inflammatory, analgesic, anxyolitic, antidepressant, antiemetic, hepatoprotector and neuroprotector.

Antibiotic Resistance Of Psychrotrophs Spoiling Raw Milk

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Background: Prolonged cold storage of raw milk favors the growth of psychrotrophic bacteria, which produce heat-resistant exoenzymes of considerable spoilage potential. We evaluated the distribution of antibiotic resistant traits among isolates originating from conventional dairy farming sytems, and compared then isolates from organic and conventionally managed farms.
Methods: Two studies were performed: 1) 60 isolates originating from farms, lorries, and silos (conventional farming) were analysed with ATB®PSE strips (BioMérieux, France); 2) The susceptibility tests were performed for 36 isolates from 6 samples (conventional farming), and for 43 isolates from 9 samples (organic farming) with 6 antibiotics in discs (Ceftazidim, Ciprofloxacin, Gentamycin, Imipenem, Minocycline, Trimethoprim+ Sulfamethoxazole).
Results: 1) Study 1: The ATB®PSE strips (designed to investigate the pseudomonal susceptibility/resistance), revealed that many psychrotrophs (ascribed to *Pseudomonas*, *Stenotrophomonas*, *Acinetobacter*, *Burkholderia* genera), besides exhibiting spoilage features were also multiresistant. Respectively, 42.3 %, 52.9 % and 94.1 % of the isolates, retrieved from farm, lorry and silo tanks, presented resistance to at least three classes of antibiotics (Microbiol. Res. 162, (2007), 115-123).
2) Study 2: The isolates retrieved from milk, from conventional dairy farms, expressed higher prevalence of resistance to 4 of the 6 considered antibiotics; contrarily to gentamycin and trimethoprim-sulfamethoxazole, for which both categories of isolates presented similar resistance frequencies.
Conclusions: The observations from Study 1 suggest an accumulation of antibiotic multiresistant traits among psychrotrophs along the cold chain of raw milk storage and transportation. In Study 2, dairy management practices seem also to affect the level of susceptibility of psychrotrophs to antimicrobial agents. However, more milk samples have to be analysed to confirm this preliminary data. The question of whether psychrotrophs spoiling raw milk may play a role as reservoir for antibiotic resistance genes needs to be considered. In our recent studies, we showed at laboratory scale, that flushing raw milk with pure N_2 gas constitutes an interesting perspective for limiting the spoilage and pathogenic potential of psychrotrophs; we believe the treatment may be of interest to control the antibiotic resistance potential of raw milk psychrotrophs as well.

Citrate Transport Mechanism In Prostate And Its Changes In Malignant Transformation. Implications For Fatty Acid Synthesis In Cancer

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Background: Prostate is a unique organ that produces, stores and releases large amounts of citrate. Excess citrate accumulation is possible because of a unique regulation of mitochondrial aconitase (m-ACNT). The level of citrate in prostatic lumen can reach 180 mM. Importantly, citrate level drops significantly when prostate becomes metastatic.
Methods: The present study was conducted on two human prostate cell lines: normal epithelial PNT2-C2 and strongly metastatic PC-3M. Citrate transport mechanisms were characterised using whole-cell patch clamp. Citrate metabolism in cancer cells was studied using spectrophotometric methods, real-time PCR and adhesion, motility and membrane secretory assays.
Results: Normal human prostate PNT2-C2 cells expressed a K^+-dependent citrate transport mechanism designed primarily to release citrate into the lumen. It was electrogenic with the estimated stoichiometry of 1 cit^{3-} : 4 K^+. Strongly metastatic prostate PC-3M cells were shown to express not only the same K^+-dependent citrate release mechanism but also electrogenic, Na^+-dependent citrate uptake mechanism. Citrate preincubation of prostate cells resulted in the increased metastatic cell behaviour in cancer cells but had no effect on normal PNT2-C2. Extracellular citrate in concentrations as low as 50 □M was sufficient to increase free fatty acids (FFA) synthesis in cancer cells. FA synthesis could be also decreased by (1) inhibiting fatty acid synthase (FAS) with cerulenin or (2) NADPH production through c-aconitase (c-ACNT)/c-isocitrate dehydrogenase (c-ICD) with oxalomalate. Intracellular Fe chelator (inhibiting c-ACNT activity) also reduced FA synthesis.
Conclusions: 1. Citrate release mechanism in prostate is electrogenic and K^+-dependent. 2. Prostate cancer cells express additionally Na^+-dependent uptake mechanism. 3. Extracellular citrate in low concentrations can be taken-up by cancer cells and increase their FA synthesis. 4. In cancer, NADPH for FA synthesis is supplied mainly by c-ACNT/cICD. 5. c-ACNT is overexpressed and upregulated in cancer through intracellular high levels of Fe.

Optimized Productions Of Recombinant Human Proteins, An Enzyme And Two Cytokines, In Fermentor Cultures Of The Yeast, *Pichia Pastoris*

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Background: The expression system of methylotrophic yeast, *Pichia pastoris*, is now widely used in order to express various proteins in host cells or to secrete them into the culture medium. By using this system, we tried to express three human proteins, bile-salt stimulated lipase (BSSL), and two cytokines, midkine (MK) and pleiotrophin (PTN), in high cell density fermentor cultures of *Pichia pastoris* to obtain the active or intact proteins for the investigations of these proteins.
Methods: Fermentation was started with 3.5 L medium in a 10 L vessel, and it was performed in various conditions. The amount of BSSL was determined by its lipase activity. The amounts of MK and PTN were determined by liquid chromatography analyses. The cell proliferation activity of MK was assayed using NIH3T3 cells.
Results: In the expression of BSSL, yeast invertase secretion signal was used. When the BSSL expression was induced by methanol at low cell density in the fermentor culture, almost no enzyme was accumulated in the medium. Therefore, the time of induction, and then the cell growth condition were studied. Finally, approximately 1 g/L BSSL was accumulated in the medium. In the expression of MK, when its own secretion signal was used, a half of secreted MK received yeast specific mannosylations. Thus, yeast α-mating factor (α-MF) secretion signal was used. MK secreted in the medium received no mannosylation, and the amount was 640 mg/L. However, about 70% of the product was truncated. Therefore, *pep4 Pichia pastoris* host was used, and MK was expressed at 20°C and at pH 3. At the end of one week induction, 360 mg/L of authentic MK was obtained. For the expression of PTN, α-MF secretion signal was also used, and the PTN was expressed in a fermentor at 20°C and at pH 5. At forth day after induction, 260 mg/L of intact PTN was obtained.
Conclusion: The expression system of *Pichia pastoris* is powerful one for the production of therapeutic proteins. As described above, three human proteins were successfully expressed in their suitable conditions, respectively. For the industrial production of recombinant therapeutic proteins, optimization of the fermentor production would sometimes be an indispensable and laborious work. Optimization processes described in detail at the meeting will be helpful for the fermentor expressions of other proteins.

Overcoming Multidrug Resistance In Human Cancer Cells By Dietary Phytochemicals

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Background: A major problem in the treatment of cancer is the occurrence of cellular resistance to cytotoxic drugs. Multidrug resistance is a phenomenon whereby tumors become resistant to chemically unrelated anticancer drugs. P-glycoprotein (P-gp, ABCB1) is the first member of the large ATP-binding cassette (ABC) transporter superfamily of membrane proteins. P-gp mediates resistance to various classes of chemotherapeutic agents including vinblastine, daunorubicin, and paclitaxel, by actively extruding the drugs from the cells. Multidrug resistance protein 1 (MRP1, ABCC1) is the second member of the ABC transporter family. Both P-gp and MRP1 act as anticancer drug efflux transporters and cause multidrug resistance. Therefore, P-gp and MRP1 are promising targets for the reversal of multidrug resistance and a better outcome of cancer chemotherapy. In this study, for the development of a safe and effective dual inhibitor of P-gp and MRP1 to overcome multidrug resistance, we investigated the effects of dietary phytochemicals on the functions of P-gp and MRP1.
Methods: The effects of dietary phytochemicals on the functions of P-gp and MRP1 were investigated using P-gp-overexpressing human carcinoma KB-C2 cells and human MRP1 gene-transfected KB/MRP cells. The effects of natural compounds found in dietary supplements, herbs, and foods such as sesame, ginkgo, soybean, and licorice were evaluated.
Results: The accumulation of daunorubicin, a fluorescent substrate of P-gp, increased in the presence of sesamin, ginkgolic acid, matairesinol, glycyrrhetinic acid, glabridin, and phyllodulcin in KB-C2 cells. Glycyrrhetinic acid and matairesinol also increased the accumulation of calcein, a fluorescent substrate of MRP1, in KB/MRP cells. KB-C2 and KB/MRP cells were sensitized to anticancer drugs by glycyrrhetinic acid, showing that glycyrrhetinic acid reverses multidrug resistance. The verapamil-stimulated P-gp ATPase activity was inhibited by glycyrrhetinic acid. Glycyrrhetinic acid stimulated the ATPase activity of MRP1.
Conclusions: These results suggest that dietary phytochemicals, such as glycyrrhetinic acid found in licorice, have dual inhibitory effects on P-glycoprotein and MRP1 and may become useful to enhance the efficacy of cancer chemotherapy.

Multidrug Resistance Inhibition in Acute Myeloblastic leukemia by Antisense Oligonucleotide

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Background: Acute myeloblastic leukemia (AML) is the most common form of acute leukemia in adults. One major problem in this disease is the emergence of leukemic blast cells that are resistant to anticancer drugs. This phenomenon is termed multidrug resistance (MDR). One cause of MDR is the expression of the MDR1 gene and its product, P-glycoprotein (Pgp). In the present study, we tried to inhibit the MDR phenotype with MDR1/mRNA/Pgp in leukemic cells using different antisense sequences and two non-viral vectors.
Methods: The Pgp expressing cell line was established from a parental K562 (Erythroleukemia) cell line with increasing concentrations of doxorubicin, and named KDI/20. In order to reverse the MDR phenotype due to Pgp expression, four different sequences of sense, antisense and one random sequence with phosphorothioate (PTO) modification (PS-ODN) against MDR1/mRNA were synthesized. They were used on the KDI/20 cells in combination with two non-viral vectors: (1) Fugene6 transfection reagent (cationic lipid) and (2) polyethylenimine (cationic polymer).The effect of PS-ODN was assessed at the cellular level by flow cytometry (for Pgp detection), and Rhodamine 123 assay (for functional assessment of Pgp) at the molecular level by RT-PCR(forMDR1/mRNAdetection) and MTT assay in order to assess the sensitivity of cells to doxorubicin.
Results: The results showed a decrease in the percentage of Pgp protein and MDR1/mRNA expression and an increase in the accumulation of Rh123 and drug sensitivity of cells to doxorubicin by antisense I and III.Also,the results showed 5 times resistancy to Doxorubicin in KDI/20 in comparision to parental K562 and cross resistance to Etoposide, Taxol and Vincristin.Antisense
Conclusion: Our data showed that antisense can reverse the MDR phenotype at the transcription level and the PEI vector is more efficient than cationic lipid. The reduction of MDR1/mRNAwas more significant than its protein reduction.

Pathogenesis Of Blood-Brain Barrier Breakdown Following Brain Injury: Windows For Therapeutic Intervention

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Breakdown of the blood-brain barrier (BBB) leading to cerebral edema is a life threatening complication in many forms of brain injury such as infarction, trauma, tumors and inflammation. In order for therapy to be effective in reducing morbidity and mortality, one must determine when to treat edema and with what agents. Increased endothelial caveolae leading to transcytosis of proteins and breakdown of tight junctions remain the principal mechanisms of BBB breakdown to plasma proteins leading to cerebral edema in brain injury. Our laboratory has characterized the time course of BBB breakdown in the rat cortical cold injury model. Our studies show that enhanced caveolae leading to transcytosis of protein occurs within minutes of the onset of brain injury and that this is associated with increased expression of caveolin-1, a major constituent of caveolae. Further the caveolin-1 is phosphorylated in the vessels showing BBB breakdown. Altered expression of tight junction proteins follows a specific sequence with transient decreases in expression of junctional adhesion molecule-1 at day 0.5, and of claudin-5 at day 2 post-injury. Occludin expression is decreased throughout the period of observation starting at day 2. Therefore, therapy to control early brain edema should target caveolae and caveolin-1 while therapy to attenuate decreased expression of occludin can be administered within days of the onset of brain injury. Endothelial-specific growth factors are emerging as a strategy to treat cerebral edema. The potent anti-leakage effect of angiopoietin-1 has the potential to reverse early BBB breakdown in brain injury.
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Influence Of The Etiology Of Liver Cirrhosis On The Response To Combined Intra-Arterial Chemotherapy In Patients With Advanced Hepatocellular Carcinoma

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BACKGROUND: We have previously reported that combined 24 hr intra-arterial chemotherapy (CIAC) prolongs the survival of patients with advanced HCC (aHCC) (World J Gastroenterol 2007 January 14;13(2):280-284). However, whether the response to CIAC varies with the etiology of liver cirrhosis (LC) underlying aHCC is still unknown in detail.
AIM: The aim of this study was to assess the influence of the etiology of LC on the efficacy of CIAC for aHCC.
METHODS: Fifty-two adult Japanese liver cirrhosis (LC) patients (45 men and 7 women) with or without aHCC were treated with CIAC between 2002 and 2007 at our hospital. All of the patients had a JIS score of 3 or 4 (Kudo M. et al. Hepatology 40: 1396-1405, 2004). Their tumors were inoperable according to computed tomography findings. CIAC (LV at 12 mg/hr, CDDP at 10 mg/hr, and 5-FU at 250 mg/4 or 22 hr) was administered via the proper hepatic artery every 5 days for 4 weeks using a catheter connected to a subcutaneously implanted drug delivery system. This chemotherapy regimen was continued for as long as possible.
RESULTS: There were 15 patients with HBV infection (B-LC group), 29 patients with HCV infection (C-LC group), and 8 patients with alcoholic cirrhosis (A-LC group). The Child-Pugh class was A for 6 out of 15 patients in the B-LC group and B for 9 patients, while the respective numbers were 14 and 15 patients in the C-LC group, 4 and 4 patients in the A-LC group. There were no patients with stage III disease, 7 patients with stage IVA disease, and 8 patients with stage IVB disease in the B-LC group, while the respective numbers were 4, 18, and 7 patients in the C-LC group, and 2, 5, and 1 in the A-LC group. There was 1 patient with tumor thrombi involving major portal vein branches in the C-LC group and 2 patients in the A-LC group. The percentage of patients with a complete or partial response after 4 weeks of chemotherapy was 0 % in the B-LC group versus 24.1 % in the C-LC group and 25.0% in the A-LC group. The survival time of the A-LC and C-LC groups was significantly longer than that of the B-LC group, with the median survival time being 647, 367, and 223 days, respectively (P<0.05 by Kaplan-Meier analysis with the log-rank test). In the A-LC group, the PIVKA-II level was significantly lower after chemotherapy compared with that before chemotherapy (p<0.05, Wilcoxon test), while the PIVKA-II level of the B-LC group was significantly higher after chemotherapy compared with that before and after chemothrapy in the C-LC group (p<0.05, Tukey's test).
CONCLUSIONS: CIAC was more effective for aHCC in patients with A-LC or C-LC compared with patients who had B-LC. Therefore, treatment for aHCC should be selected according the etiology of the underlying LC.

Influence Of Etiology On Host Immunity In Liver Cirrhosis Patients With Advanced Hepatocellular Carcinoma Treated By Combined Intra-Arterial Chemotherapy

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BACKGROUND: We have reported that combined 24 hr intra-arterial chemotherapy (CIAC) prolongs the survival of patients with advanced HCC (aHCC) (World J Gastroenterol 2007 January 14;13(2):280-284). We have shown that this therapy was more effective for aHCC in HCV-related (C-LC) or alcoholic (A-LC) liver cirrhosis (LC) patients compared with HBV-related LC (B-LC) patients (DDW 2006, LA). It has been reported that Th2 cytokines down-regulate antitumor immunity, while activation of type 1 T cells promotes antitumor immunity.
AIM: To estimate the influence of etiology on host immunity (Th1/Th2 balance) in LC patients with aHCC treated by CIAC.
PATIENTS/METHODS: Thirty-one adult Japanese LC patients with aHCC were treated by CIAC between 2005 and 2007 at our hospital. Their tumors were inoperable according to computed tomography findings. CIAC (LV at 12 mg/hr, CDDP at 10 mg/hr, and 5-FU at 250 mg/22 hr) was delivered via the proper hepatic artery every 5 days for 4 weeks using a catheter connected to a subcutaneously implanted drug delivery system. The control group was composed of 13 adult Japanese healthy volunteers (HV). Blood samples were collected from the patients in the early morning before and after chemotherapy. Flow cytometry was used to assess cytoplasmic IFN-gamma and IL-4 expression by peripheral blood CD4+ T cells, and the percentage of IFN-gamma+ and IL4- (Th1) or IFN-gamma- and IL4+ (Th2) cells was calculated.
RESULTS: Eighteen of the 31 patients had C-LC, 7 patients had B-LC, and 6 patients had A-LC. In the C-LC group, 4 out of 18 patients had a JIS score of 2, 7 patients had a JIS score of 3, 6 patients had a JIS score of 4, and 1 patinet had a JIS score of 5, while the respective numbers were 1, 2, 4, and 0 in the B-LC group or 0, 3, 2, and 1 in the A-LC group. The response rate was 27.8 % in the C-LC group and 33.3 % in the A-LC group, although it was only 14.3 % in the B-LC group. In the C-LC group, the percentage of Th1 cells before and after chemotherapy was significantly higher than in the HV group. However, there was no significant difference of the Th2 cells percentage after chemotherapy in the C-LC group, although the percentage of Th2 cells was significantly higher than in the HV group before chemotherapy ($p<0.05$ by Tukey's test). In the B-LC group, there were no significant differences of Th1 cells before or after chemotherapy compared with the HV group, although the percentage of Th2 cells before and after chemotherapy was significantly higher than that in the HV group ($p<0.05$ by Tukey's test). There were no significant differences of Th1 and Th2 cells in the A-LC group compared with the HV group.
CONCLUSIONS: These results indicate that the present therapy was more effective for aHCC in A-LC patients with normal immune function and in C-LC patients without Th2 dominance than in B-LC patients with Th2 dominance before and after chemotherapy.

Molecular Prediction Of The Therapeutic Response To Preoperative Chemotherapy With Paclitaxel In Breast Cancer

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Background: Breast cancer is considered to be relatively sensitive to chemotherapy and multiple combinations of cytotoxic agents are used as standard therapy. Chemotherapy is applied empirically despite the observation that not all regimens are equally effective across the population of patients. However, as yet there are no clinically useful predictive markers of a patient's response to chemotherapy. We report the application of large-scale genetic studies in breast cancer patients to molecular prediction of therapeutic response to paclitaxel in neoadjuvant chemotherapy.
Methods: We took core needle biopsy samples from patients with primary breast cancer before treatment and gene expression analyses were performed using two different systems, microarray and quantitative RT-PCR to identify the gene set for the prediction of therapeutic response. We selected genes for discriminating between non-responder (Grade0,Grade1b) and responder (Grade2,3) by AdaBoost machine-learning method to determine the greatest estimated accuracy between non-responders and responders.
Results: A set of genetic marker (5 genes) can predict pathologic response to preoperative paclitaxel chemotherapy with 90% accuracy. We have started independent validation of this prediction system on new cases in our hospital. I will summarize scheme of this validation trail
Conclusions: We suggest that gene expression profiling provide a strategy towards the development of personalized medicine. These kinds of findings bring oncologists one step closer to being able to select the most effective regimen (MAGIC BULLETS) for a particular individual.

Etoposide And Irinotecan Choice On The Basis Of Regional Brain's Polyamine Levels

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Background: Mammalian cells contain significant amounts of polyamines (putrescine:put, spermidine:spd and spermine:spm), which play different roles in various tissues. Polyamines are essential not only for the functions and metabolism of DNA but also for the differentiation of rapidly growing cells. Many observations show a strong positive correlation between polyamine levels, tumor growth and the effectiveness of anti-cancer drugs and have encouraged continued study of the polyamine effect in cancer chemotherapy. Analysis of Etoposide(ET) and Irinotecan's(IR) anti-cancer drug effects on the polyamine concentrations in the regional brain may be very useful for making regional specific drug choices for brain cancer treatment based on polyamine levels.
Methods: The 3 of experimental groups composed of seven rats each received intraperitoneal injections of the drug in saline solution of ET (4.0mg/kg or 8.0mg/kg) and IR (2.0 mg/kg or 8.0 mg/kg) daily for 5 days. The control group received an injection of the same volume of saline solution. All rats were euthanized with diethyl ether on the sixth day. Blood samples were taken, and the brain was immediately removed, divided ,weighed and kept in 10% trichloroacetic acid solution (TCA). Each region was homogenized and then centrifuged. The supernatants were washed with diethyl ether to eliminate the TCA in the water layer. We measured the water layer samples using HPLC to determine the concentrations of individual polyamines and total polyamines (TPA) in six regions of the brain of rats given ET and IR, and performed hematological tests on individual blood samples.
Results: ET and IR had different influences on the regional brain. As for the rat group treated with 4.0mg/kg ET, TPA decreased significantly in the thalamus and increased significantly in the diencephalon. As for the group treated with 8.0mg/kg ET, TPA decreased significantly in the hippocampus and cortex while increasing significantly in the diencephalon. On the other hand no significant change in TPA with IR was seen. Rat treated with 8.0mg/kg IR saw a significant increase in PUT and a significant decrease in SPD in the cortex.
Conclusions: We assume that ET can noticeably inhibit cell growth considering that PUT,SPD and SPM decreased in the cortex. Monitoring and comparing the polyamine concentrations of intact rats treated with these drugs, may make it possible to select treatment that target tumor-bearing regions of the brain based on their unique polyamine levels. Both anti-cancer drugs are DNA topoisomerase enzyme inhibitors, at the same time they have different pharmacologic interactions with polyamine. We compared with polyamine levels of rats treated with ET and IR, and found that IR had no influence except in the cortex.

Stimulation Of Proline Uptake And Growth Of *Escherichia Coli* CSH4 And Its Mutants Under High Salinity Through Moderate Salinity Stress Treatment

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Background: Moderate salinity stress (MSS) treatment was carried out for the activity stimulation of *Escherichia coli* and its mutants under high salinity. Aim: 1) To confirm the effectiveness of MSS treatment on proline uptake and growth 2) To compare the activity difference after MSS treatment among mutants with and without proline transporters 3) To identify the important factor in MSS solution to endure sufficient growth.
Methods: MSS treatment for *E. coli* CSH4 mutants was carried out in Davis chemically defined medium with 5 mM proline and 0.5 M NaCl at 30 ºC for 1 h. *E. coli* CSH4 mutants used in this study were CSH4 (F^- *trp lacZ rpsL thi),* JT34 (CSH4 *putP3*::Tn5), JT31 (CSH4 *putA1*::Tn5), RM2 (CSH4 (*putPA*)*101*), WG207 (CSH4 (*putPA*) *101 proU205 srl*′*300*::Tn10), WG138 (CSH4 *proP219),* WG226 (CSH4 *proP219 proU205*), WG227 (CSH4 *putA1*::Tn5 *proP219 proU205*), WG170 (CSH4 (*putPA*) *101 proP219*), and WG203 (CSH4 (*putPA*) *101 proP219 proU205*).
Results: Activities of proline uptake on $proP^+$ strains (*E. coli* K-12, CSH4, JT34, JT31, RM2, WG207) were strongly enhanced after MSS treatment, but not for $proP^-$ ones (WG138, WG226, WG227, WG170, WG203). Proline dehydrogenase encoded to *putA* supported proline uptake, *i.e.*, amounts of proline in $proP^+putA^+$ strains (K-12, CSH4, JT34) were higher than those of $proP^+putA^-$ strains (JT31, RM2, WG207). Sufficient growth after MSS treatment was observed in medium G-I containing glycine betaine (GB) for every $proP^-$ strain, while $proP^+$ strains failed to grow. High and low concentrations of proline accumulated in $proP^+$ and $proP^-$ strains during MSS treatment brought about insufficient and sufficient growth, respectively. Proline accumulated prior to GB in $proP^+$ strains interfered further uptake of GB and failed to grow in the medium. Mutant strains grew well after MSS treatment under the co-existence of cation, K^+ or Na^+, and PO_4^{3-} except for $proP^+putA^+$ strains, those of which failed to grow due to the accumulation of proline during such MSS treatment.
Conclusions: 1) Proline uptake was enhanced for $proP^+$ but not for $proP^-$ strains by MSS treatment 2) Growth of mutants strains was dependent on the amount of proline accumulated during MSS treatment 3) Growth and proline uptake of $proP^+putA^-$ strains were K^+-dependent.

Amperometric Biosensor Development And Their Clinical Application

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Background: Polyamines have important roles in biochemical processes. Their analysis in clinical diagnosis has substantial importance. In our earlier work an amperometric putrescine biosensor was successfully developed for clinical detection of bacterial infection. Elevated polyamine concentrations in human urine and serum appear to be associated with the presence of many types of cancer. The detection of diamines in clinical samples can be important for diagnosis of malignancy and for monitoring the efficiency of treatment. We made efforts to improve measuring range of the sensor.
Methods: Chemically modified electrodes and advanced electrochemical work stations were employed. Electrochemical method was used for preparation ultra thin size exclusion membrane to provided selectivity. Novel enzyme immobilization procedure was developed for getting highly active reaction layer.
Guinea pig blood and plasma samples served for spiking experiments. *In vivo* studies were also performed in abdominal tissue of anesthetized Wistar rats.
Results: *A.)* Improved biocompatible amperomertic enzyme sensor was developed. It was made of an of a flat form amperometric microcell fabricated with thin film technology on flexible Kapton® substrate, and of an improved biocatalytic reaction layer. Highly active putrescine oxidese (PUO) in reaction layer catalyzes the reaction:

$$Putrescine + H_2O + O_2 \xrightarrow{PUO} 4\text{-}Aminobutyraldehyde + NH_3 + H_2O_2$$

The platinum-working electrode detects the hydrogen peroxide produced.
Preparation of the biocatalytic enzyme- and outer protective layers was optimized for improved sensitivity and response time. A detection limit of 50 nM was achieved in pH-adjusted whole blood samples, which is below pathological levels.
B.) A novel detection principle, named periodically interrupted amperometry (PIA) that generally can be applied for improving performance of amperometric biosensors has been developed. It involves application of new amperometric working program. Using it the sensitivity of amperometric biosensors like putrescine electrode could be improved by several orders of magnitudes.
Conclusions: Two different methods were successfully used for improving the performance of amperometric biosensors. The improvements were experimentally tested measuring low level putrescine that has importance in clinical diagnosis. The measuring procedure is simple, needs small sample size or *in vivo* application is available with minimal invasion.

Properties Of The Key Reaction Steps In Demechlocycline Biosynthesis Of The Engineered *Streptomyces Aureofaciens* Strains

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Background: 6-Demethyl-7-chlortetracycline (demeclocycline; 6-DCT) and 6-demethyltetracycline (6-DMT) are produced by the mutants lacking the 6-methylation step of chlortetracycline (CTC) biosynthesis in *Streptomyces* species. To understand the pathway metabolism, we focused on two by-products, and investigated the causes of the syntheses. One of the by-products is a melanin-like pigment (MP) shown in both culture broths. Another is tetramid blue (TB) shown in the 6-DMT culture broth.
Methods: Mutants defective in the 6-DCT biosynthetic pathway were derived from *Streptomyces aureofaciens* strains, and classified by metabolites. To identify the causes of the by-product syntheses, genetic complementation by integrative transformation, gene analysis, and bioconversion analysis were performed.
Results: MP synthesis is caused by a lack of the 6-methylation step, redirecting the carbon flux from a certain intermediate in the altered CTC biosynthetic pathway to a shunt pathway leading to MP. The enzyme for the penultimate reaction step of CTC biosynthesis, designated ATC oxygenase, also takes part in the synthesis of MP. TB is a shunt product of 6-DMT biosynthesis, branched off from the labile last intermediate in the pathway. The corresponding 7-chlorinated shunt product was not shown in the 6-DCT culture broth, although the *tchA* mutants defective in the last step of the 6-DCT pathway produced it abundantly. The analysis of the *tchA* gene revealed that the gene product is a homolog of FbiB, which is known to modify a cofactor 7,8-dedimethyl-8-hydroxy-5-deazariboflavin (FO) to yield F_{420}-5,6 in *Mycobacterium*, and the *tchA* homologs are also conserved in some *Streptomyces* strains.
Conclusions: The lower substrate specificity of ATC oxygenase contributes to the diversity of the CTC-related compounds. The reactivity of the final step for adding antibiotic activity to the intermediates is crucial for the differentiations of the yield of demethyltetracyclines in *Streptomyces aureofaciens*. The *tchA* gene involved is not a special gene in the CTC biosynthetic gene cluster but a conserved gene in the *Streptomyces* genome, suggesting that the native products of CTC biosynthesis might not be antibiotics.

Reduced Vancomycin Clearance Despite Unchanged Creatinine Clearance In Patients Treated With Vancomycin For Longer Than 4 Weeks

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Background: For patients with certain types of infection such as osteomyelitis and endocarditis, vancomycin may be administrated during long-term beyond 4 weeks. Creatinine clearance-based nomograms are used routinely during the early phase of vancomycin therapy for individualizing doses. We studied whether such nomograms are also valid for patients receiving the drug for an extended period of longer than 4 weeks.
Methods: A retrospective analysis was conducted on the therapeutic drug monitoring data obtained from 85 patients who received an intermittent intravenous infusion of vancomycin. The patients were allocated to one of five groups according to the length of drug exposure: Group 1 (4 to 7 days; n = 31), Group 2 (8 to 14 days; n = 22), Group 3 (15 to 21 days; n = 13), Group 4 (22 to 28 days; n = 8) and Group 5 (longer than 29 days; n = 11). Systemic clearance of vancomycin (CL_{VCM}) and estimated creatinine clearance (CLcr) calculated by Cockcroft & Gault's formula obtained from Groups 2 through 5 were compared to those from Group 1 by Dunnet test. In addition, correlation between CL_{VCM} and the duration of vancomycin therapy was examined by Pearson's correlation analysis.
Results: Patients who had received vancomycin for longer than 4 weeks (Group 5) showed a significant ($p < 0.05$) reduction in CL_{VCM} by 50% compared to Group 1, whereas CLcr remained unchanged. In addition, a significant negative correlation was found between CL_{VCM}/CLcr ratio and dutation of vancomycin exposure ($r=-0.337$, $p<0.01$). In the analysis of patients administrated nephrotoxic agents concomitantly with vancomycin, there were no significant differences in CLcr, CL_{VCM} and CL_{VCM}/CLcr ratio between patients treated for less than 14 days and longer than 14days..
Conclusions: This study demonstrated that prolonged administration of vancomycin for over 4 weeks may result in a more pronounced reduction in CL_{VCM} than CLcr. Our data suggest that CLcr-based nomograms for individualizing vancomycin doses should be used with caution in patients who require substantially prolonged drug exposure, such as those with infective endocarditis.
Authors' disclosure statement
Reference
Nakayama H, Echizen H et al, Ther Drug Monit 2008; 30:103-107

Central Sympatholytic and Anti-arrhythmic Effects of Serotonin-1A Agonists

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Background: Psychological stressors may provoke cardiac effects ranging from mild tachycardia to ventricular arrhythmia, fibrillation and ultimately to sudden cardiac death. At present, the only class of pharmacological agents used for preventing these consequences is β-blockers acting directly on the heart. Since these drugs have a number of side effects and counter-indications, the ability to suppress potentially deleterious increase in cardiac sympathetic activity at its origin, in the brain, would be a valuable alternative. In the current study, we tested whether ventricular arrhythmias precipitated by acute stresses could be suppressed by systemic administration of 8-OH-DPAT, a 5-HT1A agonist possessing central sympatholytic properties.
Methods: The study was conducted on 33 adult male rats instrumented for telemetric recordings of ECG, body temperature and locomotor activity. In the first experiment, rats were subjected to social defeat after either 8-OH-DPAT (100 μg/kg s.c.) or vehicle. In the second experiment, prior to vehicle/8-OH-DPAT administration, animals were pre-treated with zatebradine (2 mg/kg sc.), a blocker of the pacemaker current.
Results: 8-OH-DPAT caused prolongation of basal RR interval (169±6 to 200±5 ms, $p<0.01$), increase in locomotion (10±2 to 19±2 counts/min, $p<0.01$) and hypothermia (37.9 to 36.6ºC, $p<0.05$). Subjecting vehicle-treated animals to social defeat caused shortening in RR interval, increase in locomotor activity and hyperthermia, and provoked the occurrence of ectopic ventricular and supraventricular beats; all these effects were substantially attenuated by 8-OH-DPAT. Zatebradine caused prolongation of RR interval. In zatebradine/vehicle-treated rats, incidence of ventricular and supraventricular ectopic beats during defeat increased 2.5-fold and 3.5-fold, respectively. 8-OH-DPAT administered after zatebradine completely abolished these stress-induced arrhythmias.
Conclusions: 1) Pharmacologically induced prolongation of RR interval results in an increased susceptibility to stress-induced cardiac arrhythmias, possibly due to the prolongation of the ventricular diastolic period with restored excitability; 2) Systemic administration of 8-OH-DPAT entirely abolishes these arrhythmic events, likely by suppressing stress-induced cardiac sympathetic outflow.

Immune Refocusing Technology - Improving On Mother Nature's Immunogenicity

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Background: The "potential"immunogenicity of a protein in nature is critically important to the design of future vaccines and monoclonal antibodies and yet remains a poorly understood phenomenon. Even less understood are technologies for modulating the antigenic properties while keeping the original protein conformation intact. We have found that many pathogens adopted a naturally-occuring, dis-porportionate immunogenicity through evolved immunodominance and genetic instability to mis-direct the host defense systems, a phenomena termed "Deceptive Imprinting" We have devised a way to modulate the natural antigencity of a molecule and hypothesized that dampening immunodominant subtype-restricted viral epitopes while maintaining complex conformation would allow the immune system to target other potentially more broadly protective epitopes.
Methods: To test this hypothesis the immunodominant heirarchy of the major antigenic B-cell eptiopes (in vivo and in silico) were mapped and a panel (n=10) of epitope dampened HA1 hemagglutinin (HA) subunit antigens made. Ten groups of mice (n=6) were immunized with a DNA prime and recombinant HA protein boost of wildtype (HA-wt) or HA-dampened (HA-d) antigens and the sera tested for ELISA, hemagglutination inhibition (HAI) and micro-neutralization activities against homologous and a panel of heterologous H3N2 viruses.
Results: Sera from the HA-wt and HA-d animal groups contained quantitatively similar levels of anti-HA antibodies as measured by HA ELISA (~1:300,000), however against different H3 viral subtypes showed significant qualitative HAI differences. Sera from HA-d animals were capble of protective HAI titers (>/=1:40) for viruses in the panel from 4 years prior (Pan/99) to three years ahead of that used in the vaccination. As much as 8.5-fold higher HAI titers were observed for some hetero-typic subtypes (Well.-1:10,240 vs. 1:1280) and Pan.-1:1920 vs. 1:226. Other HA-d muations when combined, demostrated more than additive HAI activity.
Conclusions: The study demonstrates that **Immune Refocusing Technology** is cable of altering the natural immunogenicity and producing a subunit antigen capable of inducing in the host new anti-viral immune responses (e.g. increased breadth and titers of protective antibody).

Optimising High Dose Melphalan In Patients With Multiple Myeloma: Preliminary Results From A Multi-Centre Trial

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Background: The aim of this study is to examine the pharmacokinetics (PK) and pharmacodynamics (PD) of total and unbound melphalan in patients with myeloma. Such studies are essential for determining the optimal dose, to avoid the profound toxicity, including cytopenia and gastrointestinal toxicity, and to ensure a good disease response.
Methods: NONMEM VI was used to develop population PK models for total and unbound melphalan from concentration-time data collected from 77 patients (36-73 years) who received a median 191 mg/m^2 dose (range: 140 – 450 mg) on a single occasion. Posterior Bayesian estimates of total and unbound area-under-the-concentration-versus time curve (AUC) were obtained and tested for significant associations with (1) Overall Disease Response, based on % change in paraprotein from diagnosis to post transplant (2) Transplant-Related Disease Response, based on % change in paraprotein from pre to post transplant, (3) the severity of mucositis, vomiting, nausea or diarrhoea (graded using CTC criteria version 3.0) and (4) the duration of hospital admission.
Results: Unbound AUC ranged from 0.95 to 6.55 mg/L.h and was significantly higher for patients who had Complete or Very Good Partial Overall Disease Response ($p < 0.05$, n = 25), severe (≥ Grade 3) mucositis ($p < 0.001$, n = 9) or nausea ($p < 0.05$, n =11) and those whose duration of hospital admission was > 22 days (the 75th percentile, $p < 0.005$, n = 18) using the Mann-Whitney test. Total AUC ranged from 6.4 to 24.6 mg/L.h and was significantly higher in patients who had severe mucositis ($p < 0.05$) and those whose duration of hospital admission was > 22 days ($p < 0.01$). Total and unbound AUC were not significantly associated with Transplant-Related Disease Response, but some PD data is missing.
Conclusions: These preliminary results suggest that melphalan pharmacokinetics (in particular, unbound AUC) is an important determinant of melphalan toxicity and efficacy. We are continuing to recruit patients and collect data (including missing data) and, eventually, we hope to devise an optimal dosing strategy for this patient group

Aminoglycosides: Deadly Bullets In The Hands Of Unexperienced

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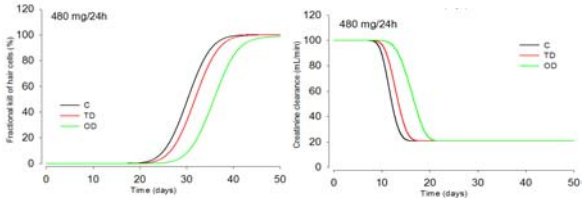
Background: Therapeutic Drug Monitoring (TDM) of aminoglycosides has been a topic during the last thirty years. There is a tendency that – because of the once daily regimen – monitoring is considered not necessary anymore. Using the model we developed for efficacy and toxicity of aminoglycosides we are able to demonstrate that optimal dosing still needs a sophisticated system of TDM.
Methods: A numerical program in Matlab is developed to run simulations for suitably chosen parameters and that can be used in TDM. The extended mathematical model that is derived incorporates the effects of aminoglycosides on bacteria, the saturable and active uptake into kidney cells, the reversible nephrotoxicity and the irreversible ototoxicity. For a continuous administration, analytical solutions are calculated for the optimal concentration in the blood for efficacy and the concentration without nephrotoxicity.
Results: From a previous model we developed it appears that the efficacy of antibiotics is not only concentration, but also dependent on the exposure time, as long as the concentration is above the MIC. This means that the AUC above the MIC is the pharmacokinetic parameter that correlates best with efficacy. In this model an optimal concentration is defined for tobramycine as a constant factor to the MIC of a specific bacteria. At this concentration (3.2 x MIC) the efficacy is optimal. The model predicts that this effect occurs when the drugs are given using a continuous infusion. This is not possible for aminoglycosides because of their toxicity. Thus aminoglycosides have to be administered intermittently.
In the figure it is demonstrated that a dosing regimen leading to adequate plasma levels leading to bacterial death causes severe nephro- and ototoxicity. For a micro-organism with a MIC of 1 mg/L a dose of 480 mg is the optimal dose in terms of efficacy. In a once daily regimen nephrotoxicity can be noticed after 15 days, whereas damage to the ear cells starts after 30 days.



Conclusion: The model can be used to design appropriate antibiotic drug regimes to combine maximal efficacy and minimal (acceptable) toxicity.

Synthesis And Biological Evaluation Of Novel Pyrazole And Pyrazolo[3,4-D]Pyrimidines As Anti-Inflammatory Agents

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Background: Non-steroidal anti-inflammatory drugs (NSAIDs) are important therapeutic agents for the treatment of pain and inflammation. Their mechanism of action involves inhibition of prostaglandin synthesis by the enzyme cyclooxygenase (COX). It has been reported that pyrazolopyrimidines inhibit COX enzymes, and therefore, may possess anti-inflammatory properties. Recently, we have synthesized novel pyrazole and pyrazolopyrimidine derivatives to investigate their anti-inflammatory effects.

Methods: A diketo-dipyrazole compound and an acid chloride of pyrazolo[3,4-d]pyrimidine 4(5H)-one were prepared via the reaction of 5-amino-4-cyano-1-phenylpyrazole with oxalyl chloride in tetrahydrofuran at room temperature. The product compounds were then reacted with a number of amine nucleophiles. The resultant novel compounds were tested in several *in vitro* assays to assess their anti-inflammatory activity. Their effects on the secretion of pro-inflammatory cytokines and neurotoxins by human THP-1 monocytic cells were studied. In addition, effects of the various compounds on the viability of human cell lines were monitored.

Results: The derivatives of pyrazolo[3,4-d]pyrimidine 4(5H)-ones displayed significant differences in their effects on the various parameters studied. Some derivatives induced significant toxic effects at concentrations above 50 μM, while other derivatives appeared to be well tolerated by the cultured cells. We identified novel compounds that were able to reduce secretion of pro-inflammatory cytokines by human monocytic cells without causing any toxic effects. These compounds also inhibited monocytic cell toxicity towards human neuronal cells. The anti-inflammatory activity was observed in the low micromolar range.

Conclusions: Some of the newly synthesized derivatives displayed anti-inflammatory properties when tested *in vitro* by using human THP-1 monocytic cells. In several cases significant toxic effects were also observed. The newly synthesized compounds may hold potential as either anti-inflammatory or anti-neoplastic agents.

Therapeutic Vaccination For Lymphomas: Challenges And Opportunities

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Immunotherapy is a promising approach for the treatment of follicular lymphoma (FL), the most common low-grade B-cell non-Hodgkin's lymphoma that is considered incurable and results in fatal outcome in the majority of patients. Immunization with customized vaccines made from the clonal tumor immunoglobulin molecule, termed idiotype, induced tumor-specific T-cell immunity in greater than 80% of patients with lymphoma. However, objective clinical responses were observed in only a minority of patients following idiotype vaccination. Although the magnitude or quality of the immune responses may be a reason for the low clinical response rate, resistance to the effector phase of the antitumor T-cell response due to immunosuppressive mechanisms in the tumor microenvironment is also thought to play a major role. Important negative regulatory pathways that inhibit T-cell function include extrinsic suppression by regulatory T cells, direct inhibition through inhibitory ligands such as PD-L1, and metabolic dysregulation of essential amino acids such as tryptophan. The relative contributions of these inhibitory processes will be reviewed and novel approaches to enhance the efficacy of therapeutic vaccination strategies in human lymphomas will be discussed.

Antibiotic Treatment For Clostridium Difficile-Associated Diarrhea In Adults

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Background: The aim of this review is to establish the efficacy of antibiotic therapy for *C. difficile*-associated diarrhea (CDAD), to identify the most effective antibiotic treatment for CDAD in adults.
Methods: Only randomized, controlled trials assessing antibiotic treatment for CDAD were included. The following outcomes were sought: resolution of diarrhea; conversion of stool to *C. difficile* cytotoxin and/or stool culture negative; recurrence of diarrhea; recurrence of fecal *C. difficile* cytotoxin and/or positive stool culture; patient response to cessation of prior antibiotic therapy; sepsis; emergent surgery: fecal diversion or colectomy; and death. For dichotomous outcomes, relative risks (RR) and 95% confidence intervals (CI) were derived from each study. When appropriate, the results of included studies were combined for each outcome, using a fixed effect model, except where significant heterogeneity was detected, at which time the random effects model was used.
R**esults:** Twelve studies (1157 participants with CDAD) were included. Eight different antibiotics were investigated: vancomycin, metronidazole, fusidic acid, nitazoxanide, teicoplanin, rifampin, rifaximin and bacitracin. In paired comparisons, no single antibiotic was clearly superior to others, though teicoplanin, an antibiotic of limited availability and great cost, showed in some outcomes significant benefit over vancomycin and fusidic acid, and a trend towards benefit compared to metronidazole. Only one placebo controlled trial was done and no conclusions can be drawn from it due to small size and classification error. Only one study investigated synergistic antibiotic combination, metronidazole and rifampin, and there was no advantage to the drug combination.
Conclusions: The only placebo-controlled study shows vancomycin's superior efficacy. However, this result should be treated with caution. The study of asymptomatic carriers also shows that placebo is better than vancomycin or metronidazole for eliminating *C. difficile* in stool during follow-up. Two goals of therapy need to be kept in mind: improvement of the patient's clinical condition and prevention of spread of *C. difficile* infection to other patients. Given these two considerations, one should choose the antibiotic that brings both symptomatic cure and bacteriologic cure. In this regard, teicoplanin appears to be the best choice. Teicoplanin is not readily available in the United States, which must be taken into account when making treatment decisions in that country

Levofloxacin For Typhoid Fever : An Unparalelled Local Success Story

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Fluoroquinolones especially Ciprofloxacin emerged as a drug of choice for treating typhoid fever during the last decade of the previous century while Levofloxacin acquired the prestigious title of Respiratory Quinolone due to its action by obtaining very high drug concentration both in the alveolar macrophages as well as lung tissue and its ability to destroy the Atypical Respiratory Pathogens. Because of a trend reported at the end of the previous century concerning decreased sensitivity of Salmonella typhi against ciprofloxacin the application to study hospitalized uncomplicated typhoid fever patients with oral levofloxacin was granted by the Faculty of Medicine University of Indonesia, Jakarta. This initial open study using 500 mg of levofloxacin once daily for 7 days consisted of 53 screened hospitalized typhoid fever patients enrolling 48 patients of them in whom 31 cases were positive by either blood culture or polymerase chain reaction or by significant serological increase of titers. From these 31 cases one cases was later excluded because of a concomittant sinus infection. After the astonishing result of resolution of fever within 2.5 days with a minimum number of side effects which were all tolerable and without any clinical relapse or S. typhi fecal carrier state one month post treatment, it was decided to continue the study and compare two regimens of treatment for uncomplicated typhoid fever. A random single blind comparative study of 500 mg ciprofloxacin twice daily for one week against once a day 500 mg levofloxacin also for one week was proposed that also received full support by the ethical committee of the Faculty. This study was carried out as a multicenter study encompassing five other medical faculties. One important criteria to mention was that all cased that did not obtain defervescence after the treatment-week with either ciprofloxacin or levofloxacin were given 500 mg ciprofloxacin as long as needed for obtaining clearance of fever.
From 212 screened hospitalized suspected typhoid fever cases, definite infection was confirmed in 110 cases consisting of 54 cases in the levofloxacin arm and 56 cases in ciprofloxacin arm.
Baseline characteristics like sex, age, length of illness and clinical score were statistically not different for both groups. Results favored better fever clearance in the levofloxacin group. Two clinical relapses occurred in the ciprofloxacin group and was confirmed by microbiological culture. Clinical adverse reactions were on the whole less in the levofloxacin group while the laboratory side effects were twice as many with more than 3 times increase of SGPT in the ciprofloxacin group of patients. The final study comparing levofloxacin IV and ciprofloxacin IV in complicated typhoid fever showed that in the toxic patient the clinical condition without resorting to the use of corticosteroids which are usually routinely administered in the toxic patient converted spontaneously. A positive imunomodulatory effect probably made a significant contribution to the reversal of the toxic delirious typhoid fever patient to become fully alert again. Perhaps at the moment we have an improved MAGIC BULLET for treatment of a life threatening condition that is especially still rampant in many countries of South and South East Asia.

Levamisole Resistance In Parasitic Nematodesinvestigated At The Molecular Level

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The nicotinic acetylcholine receptor (nAChR) is an important determinant of signal transmission at the neuromuscular junction. Helminth nAChRs are selectively targeted by many drugs such as the anthelmintic family agents imidazothiazoles (e.g., levamisole). However, the high efficacy of levamisole treatments against gastrointestinal parasitic nematodes of ruminants has been compromised by the development of resistance in field parasite populations. In order to investigate molecular mechanisms involved in levamisole resistance a candidate gene strategy has been initiated. In the free-living nematode *Caenorhabditis elegans*, the levamisole-sensitive nAChR is composed of five multi-transmembrane spanning subunits encoded by *unc-29*, *lev-1*, *unc-63*, *unc-38* and *lev-8* genes and mutants lacking one of those genes are resistant to levamisole. Here we have identified and sequenced *unc-29*, *lev-1*, *unc-63* and *unc-38* orthologs isolated from the trichostrongylid nematode *Haemonchus contortus* that is causing major economic losses to sheep industry throughout the world. Expression studies of those genes in levamisole resistant and susceptible isolates of *H. contortus* revealed specific expression of alternatively spliced RNA messenger in resistant isolates. If the alternative splicing of nAChR subunits is well documented in insects, this work constitutes to our knowledge the first report of such a phenomenon in nematodes.

Longterm Therapy Of Brain Tumors With Temozolomide: Review Of Tolerability And Efficacy In 53 Patients

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Background: Temozolomide (TZM) is a 2nd generation alkylating agent with significant efficacy for low-grade and malignant brain tumors. The drug is administered orally for 5 days every month (150-200 mg/m^2/day), using a conventional schedule. Due to TZM's excellent tolerability and lack of cumulative systemic toxicity, some patients are receiving treatment for 12 to 24 months. The efficacy, safety, and tolerability of this longterm therapeutic approach remains unclear.
Methods: We performed a retrospective chart review of all Neuro-Oncology Center patients who had undergone temozolomide chemotherapy for 12 months or longer.
Results: A total of 53 patients (median age 45 years) met the criteria; tumor types included glioblastoma multiforme (GBM; 17), oligodendroglioma (10), anaplastic glioma (12), astrocytoma (6), other glioma (7), and primary CNS lymphoma (1). Forty-one patients had received irradiation; 10 had prior chemotherapy. The median number of monthly TZM cycles was 20 (range 12-28; 38 patients ≥ 18 cycles), with a median TZM dose of 400 mg/day. Median time to progression was 34+ months (range 14 to 65+ months; 30+ months in GBM cohort), with 15 objective responses by MRI (28.3%). Toxicity included mild to moderate fatigue (98%), mild nausea (85%), constipation (70%), and grade I/II leukopenia (66%) and thrombocytopenia (47%). Of 1087 total cycles of TZM, 18 (1.7%) were delayed at least one week by treatment-related side effects. No lymphoproliferative disorders have been documented.
Conclusions: Longterm treatment with TZM is feasible, and demonstrates durable activity and acceptable toxicity in patients with gliomas, including GBM.

In Vitro* Pharmacodynamic Evaluation Of Intracellular Activity Of Antibiotics (ABs) Alone Or In Combination Against A Small Colony Variant (SCV) Of *Staphylococcus Aureus

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Background: SCVs show reduced AB susceptibility and persist intracellularly, which may cause therapeutic failures. The intracellular activities of OXA (oxacillin), FA (fusidic acid), CLI (clindamycin), GEN (gentamicin), RIF (rifampin), VAN (vancomycin), LNZ (linezolid), Q-D (quinupristin-dalfopristin), DAP (daptomycin), TGC (tigecycline), MXF (moxifloxacin), TLV (telavancin), and ORI (oritavancin), alone or in combination, were examined in THP-1 macrophages infected by a stable thymidine-dependent SCV in comparison with normal phenotype and revertant isogenic strains isolated from the same cystic fibrosis patient.
Methods: Intracellular activities were determined in THP-1 macrophages after 24 h or 72 h of exposure to ABs. Combinations were tested at fixed concentrations and then using the Fractional Maximal Effect method (FME).
Results: At C_{max}, ORI caused a 2 log CFU reduction at 24 h, RIF, MXF, and Q-D, a similar reduction at 72 h. All other ABs showed a static effect at 24 h and 1 log CFU reduction at 72 h. Dose-effect studies showed a bimodal curve with 2 successive plateaus at -0.4 and -3.1 log CFU for ORI; maximal effects of -1.1 to -1.7 log CFU for TGC, MXF, and RIF, and of ≤ - 0.6 log CFU for the other ABs. Addition of thymidine restored the SCV intracellular growth, but did not modify the AB activity except for Q-D. All drugs showed higher intracellular activity against normal or revertant phenotypes than against SCVs, except TGC and ORI.
At C_{static}, all combinations with RIF or ORI proved more active (in particular OXA, GEN and MXF). At C_{max}, all combinations with RIF were less active than RIF alone, while combinations of ORI with GEN, Q-D, or RIF were more active than ORI alone. Using the FME method, RIF and ORI were synergistic at all concentration ratios investigated, ORI and MXF were also synergistic but at large ORI concentrations only. RIF and MXF were additive.
Conclusion: Intracellular SCV are poorly susceptible to most ABs, which may contribute to the difficulty of eradicating such infections. Our studies may help in selecting most active drugs or appropriate combinations to rationalize AB treatment of persistent infections involving SCVs.

Doripenem, A New Carbapenem: Optimizing Dose To Treat Increasingly Resistant Gram-Negative Pathogens.

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Background: The growing number of infections due to multi-drug resistant (MDR) Gram-negative pathogens such as *Pseudomonas aeruginosa* has prompted exploration for new, highly effective anti-infective agents as well as the more rational use of available anti-infectives. Doripenem is a new carbapenem with increased in vitro microbiologic potency, low potential for seizure induction, and prolonged stability in solution.
Methods: These features allow for increased dose and prolonged infusion duration, which optimizes the carbapenem time-dependent pharmacokinetics and pharmacodynamics (PK/PD), and hopefully increases clinical efficacy against serious Gram-negative infections while minimizing the emergence of resistance.
Results: Doripenem has been studied at 500 mg infused over 1 hour Q8H for the treatment of complicated urinary tract infections, intraabdominal infections, and nosocomial pneumonia. A prolonged infusion of the 500 mg dose over 4 hours has been studied in later-onset ventilator-associated pneumonia, acknowledging that pathogens with higher carbapenem MICs are more likely in this patient population. Additional studies are underway with a 1-gram dose infused over 4 hours in patients with nosocomial pneumonia, including ventilator-associated pneumonia, who are at particular risk of carbapenem resistant *P. aeruginosa* infection. Exploiting the safety of high dose doripenem and the longer stability in solution, strategies to increase the time over MIC of this highly potent carbapenem, theoretically allows for the treatment of infections due imipenem-resistant pathogens where other therapeutic options are severely limited.
Conclusion: In summary, doripenem holds promise as a new "magic bullet" for the treatment of infections involving MDR Gram-negative pathogens, particularly *Pseudomonas aeruginosa*.

Authors' disclosure statement: Susan C. Nicholson and Janet A. Peterson are employees of Ortho-McNeil Janssen Scientific Affairs, LLC.

Sugammadex A Novel Cyclodextrin For The Reversal Of Neuromuscular Blockade

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Background: Following surgery, reversal of the non-depolarizing effects on skeletal muscle relaxation is facilitated by the administration of an acetylcholinesterase inhibitor. Although effective, this method of reversal presents issues including muscarinic adverse effects and administration timing. Sugammadex is a modified gamma-cyclodextrin compound pharmacologically unrelated to the acetylcholinesterase inhibitors. Currently, pharmaceutical uses of cyclodextrins have been primarily limited to excipients in drug formulation. Sugammadex is a selective relaxant binding agent, which forms a 1:1 complex with steroidal non-depolarizing neuromuscular blockers within the plasma. This results in lowering the availability of the neuromuscular blocker at the nicotinic receptor. Unlike acetylcholinesterase inhibitors, prior studies have demonstrated that sugammadex can elicit rapid reversal during profound neuromuscular block.
Methods: Using acceleromyography, we describe reversal in two surgical cases requiring pharmacologic reversal of neuromuscular blockade. The primary outcome measure was a train of four ratio (TOF) of 0.9. In the first case, the acetylcholinesterase inhibitor neostigmine antagonized the effects of non-depolarizing blockade with rocuronium and vecuronium. In a subsequent case, sugammadex was administered for reversal of rocuronium-induced neuromuscular blockade.
Results: Both methods employed for reversal were effective. The TOF ratio of 0.9 was achieved 4 min, 41 sec after administration of neostigmine following partial spontaneous recovery of TOF ratio (0.5) and 1 min, 14 sec with sugammadex following partial spontaneous recovery of TOF ratio (0.25). Additionally, after a TOF ratio of 0.9 was achieved in the neostigmine-treated patient, there was a period of 4 min, 15 sec when the ratio decreased to below the desired level of 0.9. In contrast, the TOF ratio was maintained at 0.9 or higher in the sugammadex-treated patient, once a level of 0.9 was obtained.
Conclusions: Sugammadex provides novel approach for the reversal of amino-steroidal induced neuromuscular blockade. In contrast to acetylcholinesterase inhibition, sugammadex does not increase endogenous levels of acetylcholine or require the co-administration of anticholinergic agents.
Authors' disclosure statement: The authors and the Mayo Clinic College of Medicine have conducted sponsored clinical research studies of sugammadex for Organon/ Schering Plough.

3-O-Methylfunicone, A Fungitoxic And Antitumor Extrolite Produced By *Penicillium Pinophilum*

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The soil fungus *Penicillium pinophilum* has been described as an ecological antagonist and a mycoparasite of the widespread plant pathogen *Rhizoctonia solani*. Its antifungal properties have been reported in relation to the production of 3-O-methylfunicone (OMF), an extrolite characterized by liquid cultures of an isolate of this species (LT4) that is patented by CRA (Italian patent no. 01308189, 2001). OMF is able to inhibit hyphal growth of *R. solani* and other plant pathogenic and dermatophytic fungi *in vitro* at a concentration of 0.1 mg/ml. Fungitoxicity is also evident after treatment of actively growing cultures, where the integrity of the hyphal structure appear to be compromised, cells collapse, and their membranes are degraded. In addition, antiproliferative and pro-apoptotic properties have resulted on several human tumour cell-lines (Hep-2, HeLa, MCF-7, A549, A375M). Particularly, the compound is responsible of a cytostatic effect associated to evident morphological changes and modifications in the organization of tubulin fibres. In fact, chemical structure of OMF is based on a substituted □-pyrone ring connected to a tri-methoxylated aryl nucleus; this moiety, which also characterizes other known antitumor compounds such as combretastatin, staganacin, the podophyllotoxins, and the chalcones, is thought to interact with tubulins by binding at their sulphydryl groups. OMF also affects expression of genes involved in the cell cycle, which is arrested in the G1 phase, with a significant increase in p21 mRNA expression, and a decrease in cyclin D1 and Cdk4 mRNA. Apoptosis induction has been confirmed by DNA-laddering, annexin assay, and cytofluorimetric analysis of the DNA content of the sub-G1 fraction; the triggered apoptotic pathway is p53 independent. In MCF-7 cells, pro-apoptotic effects also depend on a down-regulation of tumor-associated antigens, such as survivin and hTERT. Finally, anti-metastatic effects may derive by the ability of OMF to affect cell motility, in connexion with down-regulation of □v□5 integrin and inhibition of matrix metalloproteinases. The reported effects of OMF are indicative of a potential for the development of a new agent for cancer chemotherapy.

Hormone-Immunotherapy Significantly Prolongs Clinical Benefit And Median Overall Survival Of Metastatic Endocrine Dependent Breast Cancer Patients

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Background: Metastatic disease from breast cancer is incurable. In endocrine-dependent patients antiestrogens are commonly administered as first and second line therapy. Regrettably, tumour growth becomes resistant to this relatively well tolerated therapy. In the past, beta-interferon was added to tamoxifen to induce estrogen receptor enhancement. However, clinical data did not significantly confirm experimental data. In mice, interleukin-2 added to tamoxifen increased their mutual antitumor activities. Nevertheless, no effective clinical application has been developed. We started an exploratory clinical trial based on the association of these immunostimulating cytokines with antiestrogens for first line salvage therapy of hormone dependent metastatic breast cancer.
Methods: Thirty three consecutive breast cancer patients with distant metastases, most of them with involvement of multiple organs, were studied for responsiveness to treatment with first-line salvage anti-estrogen therapy, combined with beta-interferon and interleukin-2 immunotherapy. Clinical response and survival were compared with that of 30 consecutive historical control patients treated with anti-estrogen therapy alone.
Results: Controls showed, as expected, a median duration of response, a median survival time after treatment, and after diagnosis of distant metastases, of 16, 31 and 34 months, respectively. After a mean follow-up of 74 ± 39 months (range 24-209) from the beginning of first line antiestrogen salvage therapy, the interval times in the studied patients were 33 ($p<0.001$), 74 ($p<0.001$) and 79 ($p<0.001$) months. One long-term survivor appeared to be cured after 155 months from the time of diagnosis with multiple bone metastases. Eleven (33%) of the patients treated with beta-interferon and interleukin-2 have survived.
Conclusion: These data suggest that immunotherapy, given in an outpatient setting in addition to conventional antiestrogen salvage therapy, is very well tolerated and provides an important benefit in endocrine-dependent metastatic breast cancer.

Corpora Non Agunt Nisi Fixata: Tailored Drug Targeting By Intelligent Active Principles Or Intelligent Transporters, State Of Art And Our Experience

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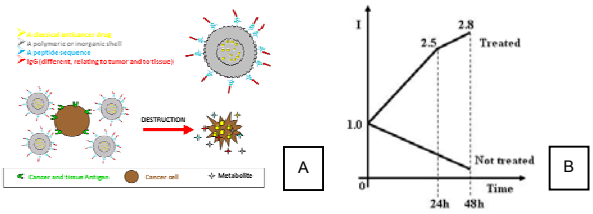
Background: Matter: insufficient therapeutic index and too big gap between research and clinic for certain diseases. Aims: integrate different scientific knowledge (molecular interaction maps, peptide interfering with protein-protein interaction, nanotechnology) to obtain smart active drugs or smart transporters of active drugs saving healthy cells and killing altered ones.
Methods: 1) synthesis of inorganic nanoshells incorporating commercially available drugs, functionalization with docking peptide, binding with specific antibody. 2) serum derivative given *in vivo* to animals and then to patients (pz), 2 phials/die for 10 days (case A orthopaedics 20 pz, case B surgery 339 pz, case C leprosy 20 pz). *In vitro* every 24h in monoculture of fibroblasts and fibrosarcoma and in co-culture 1:1 of them. Number of each cell line was registered for monoculture and ratio between normal and cancer cells was considered for co-culture (**I**).
Results: 1) final drug-delivery construct in fig 1A (not scale). 2) 100% atoxic in animals and pz evident by unaltered haematic parameters and absence of reactive effects. Anti-inflammatory, anti-exudative, anti-infective, antalgic and healing activity in 100% case A , 89% case B, significant improvement in 100% case C. *In vitro* original activity: no difference between treated and not treated cells for monocultures, whereas inversion in ratio (**I**) was clear in co-culture as shown in fig 1B.
Conclusions: 1) we intend to rapidly commercialize our intelligent drug delivery system born from interdisciplinarity 2) strict interaction between clinic and research gave a promising intelligent drug, but we need enlightened scientists of laboratories to go ahead.



Authors' disclosure statement: This presentation both gives a general view of the art in tailored therapies, especially in cancer, and in part contains our novel and unpublished data. A clarification: we are working without any specific funding, also with our private money, often adopting unconventional and simple reasoning, against the indifference and unintelligible hostility of many colleagues and superiors, but we are sure we must use all the knowledge, even not standard, against still incurable diseases. We invite enlightened scientists to collaborate for a serious, scientific and exciting research. Thanks.

The Potential Of Oxoglaucine, A New Antiviral Compound, To Inhibit Enterovirus Replication When Applied Alone Or In Combination

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Background: Despite the fact that enteroviruses are implicated in a variety of diseases, there is no drug for clinical use yet. A clear need exists for development of new antivirals and for new approaches for application of the already known ones. Aims: 1) To characterize the antiviral parameters *in vitro* of oxoglaucine, a novel inhibitor developed in our laboratory. 2) To combine oxoglaucine and disoxaril in search of a synergistic combination.
Methods: The cytopathic effect inhibition test and the plaque-inhibition assay were used for evaluation of the antiviral effect *in vitro*. The mode of action was revealed by time-of-addition tests. Resistant progeny was derived by serial passages in the presence of the compound. The combined antiviral effect *in vitro* of oxoglaucine and disoxaril was determined by the 3D model for analyzing drug interactions of Prichard and Shipman.
Results: Oxoglaucine inhibited the replication *in vitro* of 16 enteroviruses exerting a strong antiviral effect on all of them with a selectivity index greater than 100 for the most of the tested viruses. Time-of-addition study showed that the susceptible period was the latent and the lag phase of the virus cycle. Resistant virus strains were derived for poliovirus type 1 and the six coxsackie B viruses. A correlation was established between the sensitivity and the necessary number of passages in the presence of the compound for selection of resistant progeny. Reversion to sensitivity occured when the selective pressure of oxoglaucine was diminished. Evaluation of the combined effect of oxoglaucine and disoxaril revealed that the effect was additive and at some dose combinations it was moderately synergistic.
Conclusions: 1) Oxoglaucine is a newly developed antiviral compound with a strong and selective antienterovirus activity. 2) Viruses which reveal the greatest sensitivity to oxoglaucine develop most rapidly resistant progeny. 3) The combination of oxoglaucine and disoxaril is additive with areas of moderate synergy. 4) There is a promise that oxoglaucine, alone or in combination with other antiviral agents, could be developed to a "magic bullet" for the treatment of enterovirus infections.

Modulation Of The Structure Of A Lipid Membrane For Selective Interactions With A Drug

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This presentation will show how the structure of a lipid film can be modified to respond selectively to a specific drug. For this purpose, microporous filters composed of glass fibers were used as supports for the stabilization of lipid films. The lipid films were formed on the filter by polymerization using UV radiation. Methacrylic acid was the functional monomer, ethylene glycol dimethacrylate was the crosslinker and 2,2-azobis-(2-methylpropionitrile) was the initiator. Proteins [i.e., enzymes (i.e.,acetylcholinesterase, urease, etc) and natural receptors (ABP1, etc)] were incorporated within this mixture prior to polymerization and the results showed that these proteins retain their activity for repetitive uses. This method for preparation of stabilized lipid membranes was investigated using Raman spectroscopy. The results have indicated that the kinetics of polymerization are completed within 4 hours.
These lipid films were used to study the interactions of several drugs (i.e., atenolol) and other compounds.(i.e., sweeteners) with bilayer lipid membranes.(BLMs). The interactions of atenolol and artificial sweeteners with BLMs produced transient electrochemical ion current signals that reproducible appeared within a few seconds after the exposure of the membranes to the drug.or the sweetener. The current signals were related to the concentration of atenolol (20 to 200 μM) in electrolyte solution ssDNA incorporated into BLMs can interact with atenolol, and decreased the detection limit of this drug by one order of magnitude. The oligomers used were single stranded deoxyribonucleic acids: thymidylic acid icosanucleotide terminated with a C-16 alkyl chain to assist incorporation into s-BLMs (5'-hexadecyl-deoxythymidylic acid icosanucleotide, dT_{20}-C_{16}). The selectivity of the lipid films for the direct interaction with a drug can only partly be modulated by modification of the composition of the lipid film.
Further results will also present the interactions of lipid films with incorporated artificial receptors (i.e., resorcin[4]arene or methoxy-calixerene receptor) with doping materials (i.e., adrenaline, dopamine and ephedrine). These BLMs modified with the resorcin[4]arene receptor were used as sensors for the direct electrochemical sensing of these stimulating substances. The interactions of these compounds with the lipid membranes were found to be electrochemically transduced in the form of a transient current signal with a duration of seconds, which reproducibly appeared within a few s after exposure of the membranes to these doping materials.. The selectivity of response could be modulated with an alteration of the structure of the artificial receptor
The mechanism of signal generation was investigated by differential scanning calorimetric studies. These studies revealed that the adsorption of the receptor is through the hydrophobic tails of the receptor, whereas hydrophilic groups of the receptor were directed towards the electrolyte solution enhancing the ion transport through the lipid membranes. Raman spectroscopy was further used to investigate the location of the proteins into the structure of the lipid films and how are attached with the bilayer lipid membranes. This will allow the practical use of the techniques for the preparation of stable lipisomes with an incorporated drug that can selectively b e directed to a specific target.

A Novel And Effective Antibody Therapy For Brain Infarction Targeting Alarmin/HMGB1

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Background: The high mobility group box-1 (HMGB1), originally identified as an architectural nuclear protein, exhibits an inflammatory cytokine-like activity in the extracellular space. HMGB1 is released from necrotic cells and activated macrophages in different manners. The cytokine profile of HMGB1 has shed light on the role of nuclear proteins and promoted the studies on roles of this unique factor in different disease conditions such as sepsis, acute lung injury, ischemic liver injury and arthritis. In the present study, we examined the possible involvement of HMGB1 in the development of brain infarction in middle cerebral artery occlusion (MCAO) rats using neutralizing anti-HMGB1 monoclonal antibody (mAb).
Methods: MCAO was performed by the insertion of silicone-coated 4.0 nylon thread from the bifurcation of the internal and external carotid arteries under anesthesia. After 2 hour occlusion, reperfusion was started. Intravenous injection of mAb was done twice immediately and 6 h after reperfusion.
Results: Treatment with neutralizing anti-HMGB1 mAb remarkably ameliorated brain infarction induced by 2-hour MCAO in rats**,** even when the mAb was administered after the start of reperfusion. Consistent with the 90 % reduction in infarct size detected by TTC staining, the accompanying neurological deficits in locomotor function were significantly improved. Anti-HMGB1 mAb inhibited the increased permeability of the blood-brain barrier, the activation of microglia, the expression of TNF-alpha and iNOS and the morphological changes in BBB at electron microscopic levels, whereas it had little effect on blood flow.
Conclusions: These results as a whole indicate that HMGB1 plays a critical role in the development of brain infarction through the amplification of plural inflammatory responses in the ischemic region and could be an outstandingly suitable target for the treatment. Intravenous injection of neutralizing anti-HMGB1 mAb provides a novel therapeutic strategy for ischemic stroke and may make an innovation in the treatment.

Cryoimmunology Induced After Reimplantation Of Malignant Bone Tumor Treated With Liquid Nitrogen

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Background:1) In the laboratory, systemic antitumor responses following reimplantationof tumor treated with liquid nitrogen +/- dendritic cells were evaluated by measuring immune reactions and counting distant metastases in mouse osteosarcoma models. 2) In the clinical setting, immunological cytokines were measured after reconstruction with liquid nitrogen-treated autografts.
Methods: 1) LM8 mouse osteosarcoma cells were implanted subcutaneously into C3H mice. After two weeks, tumors were treated with a) excision (E) (n=15); b) liquid nitrogen (LN) (n=15); or c) liquid nitrogen plus dendritic cells (DCs) (n=15). Four weeks later, serum IFN-γ levels, lung metastases area, and CD8(+) T-lymphocyte counts in lung metastases were measured. 2) IFN-γ and IL-12 levels were measured in blood samples collected before surgery and one and three months after surgery from 23 patients with malignant bone tumors treated with tumor-bearing autograft frozen by liquid nitrogen.
Results: 1) Mean serum IFN-γ level was significantly higher in DCs (118.29 ± 5.71; $p<0.01$) than in both LN (37.22 pg/ml ± 2.74) and E (8.25 ± 2.76); LN was significantly higher than E ($p < 0.05$). Mean metastasis area was significantly smaller in DCs (5.39 ± 1.49; $p < 0.01$) than in both LN (13.21m^2 ± 2.59) and E (24.12 ± 3.60); LN was significantly smaller than E ($p < 0.01$). Mean number of CD8(+) T-lymphocytes was significantly higher in DCs (8.23 ± 2.56; $P < 0.05$) than in both LN (2.46 cells/mm^2; ± 0.57) and E (0.64 ± 0.56); LN was significantly higher than E ($p < 0.01$). 2) Mean INF-γ relative concentration of one month after and three months after against before surgery were 149.7 and 268.3%, and mean IL-12 relative concentration were 170 and 432.2%, respectively. Composite values for all 23 patients showed progressive increases in INF-γ and IL-12 levels one and three months after surgery.
Conclusions: 1) Treatment of tumor tissue with liquid nitrogen can activate the immune system and inhibit metastatic tumor growth. Dendritic cells enhanced immunological activity synergistically. 2) Patients with malignant bone tumors who received autografts treated with liquid nitrogen showed evidence of immune system activation. 3) These responses suggest that liquid nitrogen treatment of bone tumors may offer certain unique benefits.

Effect Of Insulin Resistance On In-Stent Restenosis After Coronary Stenting In Type 2 Diabetic Patients

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Background: Metabolic syndrome is related to cardiovascular diseases. Insulin resistance is one of causes for metabolic syndrome. The aim of this study was to elucidate the mechanism of the effect of insulin resistance on in-stent restenosis after coronary stenting in patients with type 2 diabetes mellitus.
Methods: A prospective, randomized trial, involving 54 type 2 diabetic patients referred for coronary stenting who were randomly assigned to either the control or the pioglitazone group, was performed. Quantitative coronary angiography was performed at study entry and at six months follow-up. Endothelial nitric oxide Synthase (eNOS), tumor necrosis factor α, interleukin-6, leptin, and adiponectin were measured at study entry and at six months follow-up.
Results: 28 patients were randomly assigned in the control group and 26 patients were assigned in the pioglitazone group. There were no significant differences in glycemic control levels or in lipid levels in the two groups at baseline or at follow up. Insulin, homeostasis model assessment insulin resistance, eNOS, and leptin at follow up were significantly reduced in the pioglitazone group compared with in the control group. The late luminal loss and in-stent restenosis were significantly less in the pioglitazone group than in the control group. Multiple regression analysis showed that leptin independently correlated with late luminal loss.
Conclusions: The treatment with pioglitazone in type 2 diabetic patients significantly reduced leptin. This decreased leptin improved insulin resistance and endothelial function with the reduction of insulin. The improved endothelial function affected the reduction of in-stent restenosis.

An Arabidopsis Transcription Factor, AtNFXL1 Gene Negatively Regulates *Fusarium* Phytotoxin Trichothecene-Induced Defense Response

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Background: Phytopathogenic *Fusarium* species produced trichothecene family of phytotoxins, which function as a virulence factor during infection of plants. Trichothecenes are known to act as translational inhibitors in eukaryotic ribosomes. Recently, we revealed that some trichothecenes such as T-2 toxin induce a programmed cell death. It is likely that trichothecene-induced cell death is contributing directly to virulence of necrotrophic fungi.
Methods: *Arabidopsis thaliana* (ecotype Columbia; Col-0) plants were gown on MS agar medium with or without trichothecenes in a growth chamber (a 16-h light / 8-h dark cycle at 22 °C) after a 3-d vernalization period. A T-DNA insertion mutant (*atnfxl1-1*) of *AtNFXL1* (CS24940) was obtained from the *Arabidopsis* Biological Resource Center. The microarray experiment was carried out using the Agilent *Arabidopsis* 1 Oligo Microarray SA and SAG levels were determined using HPLC.
Results: In *Arabidopsis*, expression of *AtNFXL1*, a homologue of the putative human transcription repressor *NF-X1*, was significantly induced by application of type A trichothecenes, such as T-2 toxin. An *atnfxl1* mutant growing on medium lacking trichothecenes showed no phenotype, whereas a hypersensitivity phenotype was observed in T-2 toxin-treated *atnfxl1* mutant. Microarray analysis indicated that several defense-related genes (i.e. *WRKYs, NBS-LRRs, EDS5, ICS1,* etc.) were upregulated in T-2 toxin-treated *atnfxl1* mutant compared to wild type. Enhanced salicylic acid (SA) accumulation was observed in T-2 toxin-treated *atnfxl1* mutant, which suggests that *AtNFXL1* functions as a negative regulator of these defense-related genes via an SA-dependent signaling pathway. We also found that the expression of *AtNFXL1* was induced by SA. Moreover, the *atnfxl1* mutant was less susceptible to a compatible phytopathogen, *Pseudomonas syringae* (*Pst* DC3000).
Conclusions: Our results indicate that *AtNFXL1* plays an important role in the trichothecene response, as well as general defense response in *Arabidopsis*.
Authors' disclosure statement:
Furthermore, several components of the AtNF-XL1-containing complex will be discussed.

Brain's Immune Cells And Anti-Inflammatory Effects Of Neuropeptides

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Background: There has been an explosion of interest in microglia, the immune cells of the central nervous system (CNS), over the past few years. They express various receptors for neurotransmitter or neuropeptides. In responding to these neurotransmitters or peptides, microglia show chemotaxis, release different kinds of cytokines and trophic factors, or attenuating excess release of cytokines induced by bacterial toxins. Many neuropeptides are produced in the brain during trauma and stroke, and are regarded as mediators of inflammation. Since a number of receptors for neuropeptides are also expressed in glial cells, they are likely to play an important role in the brain via various neuropeptides.
Methods: Primary glial cells were isolated from mixed cultures of cerebrocortical cells in postnatal day 1-3 Wistar rats or C57BL/6 mice (Kyudo, Kumamoto, Japan). Motility of microglia under the control of temperature and gas was monitored with time lapse video microscopy system (Nikon INSTEC, Fukuoka, Japan). Chemoaxis was tested using a 48-well microchemotaxis Boyden chamber (Neuroprobe, Bethesda, MD). Assay of tumor necrosis factor-□ (TNF-□ and prostaglandin E2 (PGE2) was done using ELISA and EIA kit. Expression of NGF was tested by SYBR green-based real-time quantitative RT-PCR.
Results: Some of the neuropeptides, such as bradykinin, endothelin, vasopressin and galanin, increased microglial motility and chemotaxis. Among neuropeptides, bradykinin (BK), a mediator of pain and inflammation and is produced at the site of injury, had neuroprotective effect. They inhibited lipopolysaccharide (LPS)-induced TNF-□ release. As a mechanism of such negative feedback system, production of PGE2 was observed. In addition, BK and endothelin up-regulated the production of neurotrophic factors such as nerve growth factor (NGF) in astrocytes.
Conclusion: These results suggest that some neuropeptides may have anti-inflammatory and neuroprotective effects through multiple functions on immune cells in the brain. These observations may help to understand the paradox on the role of neuropeptides in the central nervous system and may be useful for therapeutic strategy.

Combined Therapy With Pitavastatin And Eicosapentaenoic Acid Improve Platelet Activation Markers In Hyperlipidemic Patients With Type 2 Diabetes Mellitus

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Background: Platelet-derived microparticles (PDMP) play an important role in the pathogenesis of diabetic vasculopathy, and statins or eicosapentaenoic acid (EPA) have been shown to have a beneficial effect on atherosclerosis in hyperlipidemic patients. However, the influence of EPA and statins on PDMP and adiponectin in atherosclerosis is poorly understood. We investigated the effect of pitavastatin and EPA on circulating levels of PDMP and adiponectin in hyperlipidemic patients with type 2 diabetes.
Methods: One-hundred and ninety-one hyperlipidemic patients with type 2 diabetes were divided into three groups: group A received pitavastatin 2 mg once daily (n=64), group B received EPA 1,800 mg daily (n=55), and group C received both drugs (n=72). PDMP and adiponectin were measured by ELISA at baseline and after 3 and 6 months of drug treatment.
Results: 30 normolipidemic patients were recruited as healthy controls. PDMP levels prior to treatment in hyperlipidemic patients with diabetes were higher than levels in healthy controls (10.4 ± 1.9 vs. 3.1 ± 0.4 U/ml, p<0.0001), and adiponectin levels were lower than controls (3.20 ± 0.49 vs. 5.98 ± 0.42 μg/ml, p<0.0001). PDMP decreased significantly in group B (before vs. 6M, 10.6 ± 2.0 vs. 8.0 ± 1.7 U/ml, p<0.01), but not in group A (before vs. 6M, 9.4 ± 1.9 vs. 9.6 ± 1.7 U/ml, not significant). In contrast, group A exhibited a significant increase in adiponectin levels after treatment (before vs. 6M, 3.29± 0.51 vs. 4.16 ± 0.60 μg/ml, p<0.001). Furthermore, group C exhibited significant improvement in both PDMP and adiponectin levels after treatment (PDMP, before vs. 6M, 11.2 ± 2.0 vs. 4.5 ± 2.7 U/ml, p<0.001; adiponectin, before vs. 6M, 3.24 ± 0.41 vs. 4.02 ± 0.70 μg/ml, p<0.001). Reductions of PDMP in combined therapy were significantly greater than those observed with EPA alone (p < 0.05 by ANOVA). In addition, soluble CD40 ligand exhibited almost the same change as PDMP in all therapy groups.
Conclusions: These results suggest that pitavastatin possesses an adiponectin-dependent antiatherosclerotic effect, and this drug is able to enhance the anti-platelet effect of EPA. The combination therapy of pitavastatin and EPA may be beneficial for the prevention of vascular complication in hyperlipidemic patients with type 2 diabetes.

Serotonin Bridge Of 5-HT2C - 5-HT1B Receptor Over Appetite

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Background: Serotonin (5-hydroxytryptamine; 5-HT) 2C receptors reportedly mediate the appetite-suppressing effects of 5-HT drugs such as m-chlorophenylpiperazine (mCPP) and fenfluramine. Fluvoxamine, a selective serotonin reuptake inhibitor, is used for the treatment of binge eating disorder. Aims: 1) To determine the mechanisms by which fluvoxamine exerts appetite suppressing effects. 2) To determine the pharmacologic interactions of 5-HT2C and 5-HT1B receptors. 3) To propose a pathogenesis of fluvoxamine-responsive binge eating syndrome.
Methods: At first, male 5-wk-old C57BL6J mice were deprived of food for 23 h and were injected intraperitoneally (i.p.) with saline or SB 242084 (0.5-2.0 mg/kg), a selective 5-HT2C receptor antagonist. Thirty minutes later, animals were injected with saline or fluvoxamine (3–30 mg/kg) i.p., and after a further 30 min they were given chow pellets; intake of pellets was measured for the next 2 h. In the second experiment, animals were deprived of food for 23 h and were injected i.p. with saline or SB 242084 (2 mg/kg) plus SB 224289 (5 mg/kg), a selective 5-HT1B receptor antagonist. Thirty minutes later, animals were injected i.p. with saline or fluvoxamine (10 mg/kg), and after a further 30 min they were given chow pellets; intake of pellets was measured for the next 2 h. In the third experiment, animals were deprived of food for 23 h and were injected i.p. with saline or CP94253 (2.5-10 mg/kg), a selective 5-HT1B receptor agonist. Thirty minutes later, they were given chow pellets and intake of pellets was measured for next 2 h. Finally, animals were injected i.p. with saline or SB 242084 (2 mg/kg). Sixty minutes later, animals were decapitated and determined hypothalamic 5-HT1B receptor expression.
Results: Fluvoxamine, in the presence of SB242084, exerted appetite-suppressing effects while fluvoxamine or SB242084 alone has no effect. The appetite-suppressing effects were attenuated in the presence of SB224289. CP94253 exerted appetite-suppressing effects. SB242084 increased the expression of hypothalamic 5-HT1B receptors.
Conclusions: 1) Fluvoxamine and inactivation of 5-HT2C receptors exert feeding suppression through activation of 5-HT1B receptors. 2) Pharmacologic inactivation of 5-HT2C receptors increases the expression of hypothalamic 5-HT1B receptors. 3) Fluvoxamine-responsive binge eating syndrome may result from a perturbation of 5-HT2C receptor signaling.

LC-MS/(MS) Strategies For Increased Metabolite Coverage In Metabolomics

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Background: Metabolomics is the global and untargeted measurement of as many metabolites as possible in a given biological system. There is no one single analytical technique available to measure all the metabolites in a complex system such as a cell, however, liquid chromatography mass spectrometry (LC-MS) is establishing it self as the preffered metabolomics analytical technique. We have explored the possibility to use ultra high pressure chromatography and multiple ionization strategies for the mass spectrometry analysis in order to expand the number of detectable metabolites.
Methods: Methanolic extracts of human serum was used to investigate what the impact of various LC-MS strategies had on the number of detected metabolites. Evaluation of chromatography was performed through comparision of 1.7 and 3.5 uM particle size columns. Evaluation of the multiple ionization strategy was performed using an Agielnt MSD SL system with ESI and Multimode ionization sources. All LC-MS data was extracted and aligned using the freely available XCMS software (http://masspec.scripps.edu/xcms/xcms.php).
Results: Ultra high pressure liquid chromatography (UPLC) resulted in 20% more ion features detected when compared to conventional HPLC. Performing MS analysis in both positive and negative ionmode doubled the number of unique ion features compared to positive mode only, and performing APCI (atmospheric pressure chemical ionization) on the same sample resulted in an additional 20% increase in unique ion features.
Conclusions: To achieve truly non-targeted and global metabolite analysis of a complex biological system, multiple analytical techniques are needed. We have shown that sub 2uM particle size liquid chormatography paired with multiple ionization mass spectrometry can dramtically increase the number of measured metabolites.

Predicting Fluoroquinolones Ability To Kill Resistant *Streptococcus Pneumoniae* Isolates Expressing Different Genetic Mutations: Target Attainment Analysis Simulating Therapeutic Doses To Patients With Community Acquired Pneumonia

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Background: Streptococcal pneumonia is a major cause of morbidity and mortality worldwide. Fluoroquinolones are one of the mainstay drugs for treatment of these infections. However emerging resistance poses a threat to the class's future utility. This work aims to evaluate the probable efficacy of ciprofloxacin, levofloxacin, gemifloxacin, garenoxacin, and moxifloxacin in eradicating infections and preventing continued growth of resistance.
Methods: Using patient data from strep pneumonia patients in hospitals and MIC data from the CROSS study; drug regimens were compared to see the likelihood of attaining $fAUC_{0\text{-}24}$/ MIC_{all} ratios depicting goal clinical outcomes.
Results: Probability of target attainment of the fluoroquinolones against all the genetically identified resistant isolates of *Streptococcus pneuminiae* is showed in the following table:

FQs vs. *S. pneumoniae* - All Genetically Identified Resistant Strains							
Drug	**Dose**	**30**	**40**	**100**	**120**	**200**	**240**
Ciprofloxacin	250mg BD	0	0	0	0	0	0
Ciprofloxacin	500mg BD	0	0	0	0	0	0
Ciprofloxacin	750mg BD	0	0	0	0	0	0
Gare (S)	400mg QD	67.21	51.73	39.58	33.29	21.93	15.29
Gare (ELF)	400mg QD	63.44	50.05	37.77	29.19	19.88	10.81
Gemi (S)	320mg QD	46.42	35.76	4.43	3.32	0	0
Gemi (ELF)	320mg QD	71.93	51.79	34.5	29.91	4.88	4.15
Moxi (S)	400mg QD	36.8	35.67	5.57	5.48	0	0
Moxi (ELF)	400mg QD	90.89	84.11	47.12	43.96	36.56	36.22
Gati (S)	400mg QD	37.12	33.75	11.44	4.79	1.21	0
Gati (ELF)	400mg QD	51.42	41.12	28.06	27.82	1.95	1.81
Levo (S)	500mg QD	20.84	10.99	0	0	0	0
Levo (ELF)	500mg QD	26.22	15.94	0	0	0	0
Levo (S)	750mg QD	33.44	24.13	0	0	0	0
Levo (ELF)	750mg QD	37.92	30.03	3.97	0	0	0
Levo (S)	1000mg QD	34.69	5.85	3.23	0	0	0
Levo (ELF)	1000mg QD	38.22	9.09	5.49	0	0	0

Conclusions: Very few regimens are able to prevent further growth of resistant organisms when *Par*C mutations have occurred. Only garenoxacin and moxifloxacin were able to eradicate extremely resistant isolates in serum and ELF respectively.

Unexpected Effects On Angiogenesis By Type Of Low-Molecular-Weight Heparin (LMWH) And By Type Of Vehicle In Chemotherapy

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Background: Tumor growth is angiogenesis dependent**.** The study of anti-angiogenic effects *per se* in tumors is not feasible, however. We have developed a mammalian non-tumor angiogenesis assay allowing the objective quantitative assessment of relevant variables following s.c./p.o./i.v. treatment with test agents. Aims: 1) To study the effect of (i) standard heparin (UFH), (ii) different mean molecular weight (MW) fractions of UFH, (iii) tinzaparins of different MWs (produced by heparinase digestion of UFH), and (iv) dalteparin (produced by nitrous acid depolymerization of UFH). 2) To examine how low-dose metronomic-like chemotherapy affects angiogenesis induced by VEGF-A, a key heparin-binding angiogenic factor in most tumors.
Methods: The mesentery assay in adult rats was use: a pro-angiogenic agent was injected i.p. and the ensuing angiogenesis in the membranous mesentery was recorded following the s.c. injection of a heparin or the s.c. continuous-infusion chemotherapy. *Heparins*: UFH (MW ~15 kD), MW 2.5, 8, 15 and 22 kD fractions of UFH, MW 2.5 and 5.5 kD tinzaparins, and MW 6.0 kD dalteparin. *Cytostatics*: cyclophosphamide, paclitaxel, doxorubicin, cisplatin, and 5-FU. Conventional vehicles were used, some containing a radical oxygen species (ROS) scavenger. In other cases the ROS scavenger N-acetylcystein (NAC) was given concurrently with the chemotherapy.
Results: Heparin MW influenced endotoxin-induced angiogenesis strictly (r = 0.97): low MW inhibited and high MW stimulated. In VEGF-A-mediated angiogenesis, the 2.5 kD tinzaparin fraction lacked effect while the 5.5 kD tinzaparin fraction inhibited angiogenesis. Conversely, dalteparin (MW 6.0 kD) stimulated angiogenesis.
Cisplatin and 5-FU monotherapy surprisingly stimulated angiogenesis. NAC monotherapy was inert. 5-FU + NAC co-treatment reversed the significant pro-angiogenic effect of 5-FU into a significant anti-angiogenic effect. The anti-angiogenic effect of paclitaxel emerged only when the vehicle contained a ROS scavenger.
Conclusions: 1) Heparins exert MW-related effects in angiogenesis. 2) Similarly sized LMWHs, produced by diverse methods, affect angiogenesis differently. 3) Metronomic-like chemotherapy drug-specifically influences angiogenesis: some drugs stimulate while other inhibit angiogenesis. 4) ROS play a major role in angiogenesis during low-dose chemotherapy

Apoptin Magics: Bullet Or Sensor?

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Background: Apoptin, a protein derived from chicken anemia virus (CAV), induces selectively apoptosis in human tumor and transformed cells as compared to normal cells. Various gene-therapy approaches have shown the power of apoptin as a tumor-selective killer. Apoptin interaction with e.g. the anaphase promoting complex results in tumor-selective apoptosis. Furthermore, apoptin becomes phosphorylated selectively in tumor cells by a yet unknown kinase activity. These features make apoptin both a potential bullet for novel anti-cancer therapy and a sensor for identifying novel potential drug targets.
Methods: We used the transforming ability of the SV40 large T (LT) and/or small T (ST) oncogenic proteins in normal human fibroblasts to identify tumor-related pathways sensed by apoptin by co-transfection experiments. Besides, we asked whether protein-transduction domain (PTD4)-mediated delivery of apoptin protein has anticancer potential. To that end, we established several subcutaneous tumors in nude mice and applied PTD4-apoptin and controls on the tumor bearing epidermis.
Results: Systematic mutagenesis of selected protein domains in SV40 LT and ST established that both the p53- and the Rb-binding domains are not needed for activation of the tumor-selective apoptin kinase. However, both the J domain and the PP2A binding site were essential for activation of apoptin kinase. Inactivation of PP2A by means of the siRNA technology confirmed a role of this phosphatase in apoptin regulation.
In-vivo, delivery of PTD4-apoptin protein resulted in a significant growth inhibition of human hepato -, gastric - , and cervix carcinoma. Apoptosis and disruption of the tumor integrity were apparent in PTD4-apoptin, but absent in the control-treated tumors. Despite the obvious presence of apoptin protein in normal tissues, no side effects could be monitored.
Conclusion: Recent crystallographic data demonstrated that both J and PP2A-binding domains within ST are involved in its interaction with PP2A, which corroborates our data that specific suppression of PP2A results in a tumor-related kinase. PTD4-Apoptin protein delivery fulfills the requirements of a powerful and safe anticancer therapy. Overall, we conclude that apoptin is both a potential anti-cancer bullet and a valuable tumor-selective sensor, which deserves development towards the clinic.

Neurodevelopment Of Children Following Exposure To Psychotropic Medications During Gestation: A Novel Approach To Behavioral Teratology

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Background: Maternal psychopathology should be balanced against the potential teratogenic effects of psychotropic medications (PTMs), higher levels of stress hormones, neonatal syndromes, higher fetal central nervous system (CNS) transmitter activity, and child's long-term neurodevelopment. Assessing neurocognitive development of children exposed to medications in pregnancy is an inseparable part of drug safety studies in teratology, which are complicated by multiple confounders. Study design plays an integral role in controlling these factors.
Objectives: 1.To review available literature and determine the possible risks of adverse fetal/child CNS development following exposure to PTMs in utero.
2. To present a novel approach to studying behavioural teratology, while controlling for genetic and environmental factors by testing unexposed siblings from the same families.
Methods: A systematic review of studies on long-term child neurodevelopment was conducted.
IQ scores of children exposed to Venlafaxine (VLF) (n=32) were compared to their unexposed VLF siblings and healthy controls. Participants were hierarchically matched for age, gender and order of delivery. The primary outcome measure is the children's full scale IQ measured by the WPPSI-III Scales of Intelligence. Statistical analysis accounted for the clustering effect.

Results: Full Scale IQ, Performance IQ and Verbal IQ were not different between the VLF-exposed children and their unexposed siblings (105±12vs100±8; 102±15vs105±7; 105±12vs95±10). Healthy controls scored significantly higher than the VLF group in all three IQ measures (P= 0.011; 0.041; and 0.028 respectively). There were no differences between the groups in maternal IQ, socioeconomic status or children's physical characteristics.
Conclusions: In utero exposure to PTMs was not associated with impaired long-term neurocognitive development.
Assessment of siblings helps to separate the impact of antidepressant drugs from genetic and environmental factors and is strong evidence in psychotropic drug safety studies. Factors other than the antidepressant are strongly associated with children's cognitive abilities. Supported by Wyeth Pharmaceuticals.

Improving Treatment Of Urinary Tract Infections In Elderly: Roles And Functions Of Information Technologies To Suggest Antibiotic Prescriptions And To Measure Their Effects

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Background: The evolution of Escherichia coli's resistances and the development of ESBLs call for a better transmission of experts' knowledge to practising physicians; information technologies can contribute to this purpose.
Methods: To conceive Electronic Antimicrobial Drug Aid Regulation 1] Guidelines adapted to elderly are given to allow a dynamic and interactive consultation. 2] Therapy by antibiotic failures are recorded: they are related either to intolerance or to the occurrence of resistance; broad spectrum molecules prescriptions linked to patients' details are also recorded. 3] An instrument panel is designed for the follow-up of E. coli sensitivities: in a short stay unit, comparison is made by a chi-square test of 2 series including 283 and 303 colonies, each one corresponding to 12 months of urine sampling respectively in 2006 and 2008.
Results: 1] Suggestion of antibiotic: on the screen, the practitioner determines his diagnosis with signs of gravity and checks for the existence of complications: in case of emergency, a probabilistic treatment is suggested. When bacteriological data are available, suggested antibiotics are divided into 5 classes and developed into 7 tables according to the seat and gravity of the infection and various types of complications: oral or injectable form, dose and posology, length of treatment are specified, renal function is taken into account. 2] In case of adverse drug event, the imputability of the antibiotic is evaluated according to Naranjo's method; if resistance occurs, one records supporting factors related to the patient and those related to care practices. 3] The instrument panel shows a significant increase of resistance to the amoxicillin - ac. clav. ($p < 0.05$); a sensitive, yet not significant, increase of resistance to acid nalidixic (25.44% versus 32.67%) and ESBLs (2.47% versus 5.61%).
Conclusions: The computer program should make it possible to reduce unnecessary antibiotic prescriptions especially in case of asymptomatic bacteriuria, to deliver an effective treatment in case of severe infection, to prevent adverse drug events and to attenuate ecological risk linked to the increase of bacterial resistances.

New Biomarkers And Targeted Therapies For Breast Cancer

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Background: Using gene expression profiling technologies, improved methods including minimally-invasive, sensitive, specific tests could contribute significantly to cancer diagnosis, on-going monitoring, and treatment. Recently we performed a whole genome microarray study of 104 archived breast tumour and normal biopsies and, in parallel, we optimised methods enabling us to investigate global expression of extracellular mRNAs in serum (which may also be potential diagnostic/prognostic or predictive biomarkers).
Methods: RNA was extracted from 104 tumour and normal breast specimens, as well as from pre-surgery serum specimens from four recently diagnosed breast cancer patients, from their breast tumour/matched normal biopsies, and from serum specimens procured approx. 3 months post-surgery. Serum specimens from healthy age-matched volunteers acted as controls. RNA from each specimen was examined using U133 Plus2.0 arrays. Following normalisation, statistical filters were applied to identify significant differentially-expressed (DE) genes. Using univariate and multivariate methods, individual mRNAs were extensively investigated for associations with patients' clincopathological characteristics. qRT-PCR was used to validate microarray data.
Results: mRNAs were detected in all specimens. Overall, approx. 8% (of 54,675 probesets representing transcripts on the microarray) were present in serum and approx. 45% were detected in breast tissue. 7448 transcripts were DE ($P=0.0068$) between tumour and normal breast specimens and 998 ($P=0.0009$) and 1369 ($P=0.0013$), respectively, between those that resulted in relapse or death within 5 year compared to those that did not. Clinical statistical analysis identified 36 mRNAs as potential novel biomarkers; some of which tended to be associated with tumours of a basal-like profile. qRT-PCR analysis of 5 transcripts randomly selected from these validated our microarray results.
Conclusion: The implication of these novel findings is that, using microarrays, it may be possible to identify panels of intracellular and extracellular biomarkers that are useful diagnostic, prognostic and/or predictive of outcome for cancer patients.
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A Computationally Designed Mutant Of The Metallo-β-Lactamase IMP-1 Exhibits Enhanced Catalytic Efficiency

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Background: Metallo-β-lactamases (MBLs) inactivate a broad range of β-lactam antibiotics, they can be transferred horizontally, and there are no clinically useful MBL inhibitors. In addition, the evolutionary potential of MBLs is of great concern. Being able to predict their evolutionary pathways could improve our ability to effectively combat antibiotic resistance.
Methods: Computational protein design was employed to predict improved variants of the MBL IMP-1. The predictions were validated through biochemical characterization and kinetic analysis of purified enzymes overexpressed in *E. coli*. Subsequently, molecular dynamics simulations of enzyme-substrate intermediate complexes were used to decipher the molecular mechanisms for enhanced catalytic efficiency.
Results: Two mutations (F218Y and S262A) were computationally designed in IMP-1. Experimental validation showed that the single mutant IMP-1-F218Y was superior to the wild-type enzyme IMP-1: overexpression in *E. coli* yielded a higher amount of soluble protein, the protein folded well and was thermally stable, as monitored by circular dichroism spectroscopy, and it exhibited enhanced catalytic efficiencies toward the following β-lactams: nitrocefin, cephalothin, cefotaxime, ceftazidime, benzylpenicillin, ampicillin, and imipenem, mostly due to a decreased K_M. Multiple molecular dynamics simulations showed that the F218Y mutation leads to an altered hydrogen bonding pattern and to a movement of a β-hairpin loop toward the active site, which could account for the decreased K_M.
Conclusions: 1) The IMP-1-F218Y mutant was succesfully predicted to be a more efficient enzyme than the wild-type enzyme IMP-1. 2) The enzyme could easily evolve naturally through a one-nucleotide change and could lead to enhanced antibiotic resistance. 3) Successful prediction of improved MBL variants could assist the design of better antibiotics and MBL inhibitors.

Kinetic Properties Of Recombinant Factor VIIa (rFVIIa) And The Complexity Relating These To Treatment Response

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Background: Haemophilia A (HA) and B (HB) are inherited coagulation disorders caused by deficiency of factor VIII (FVIII) or IX (FIX). These patients are treated with replacement therapy with FVIII or FIX. Approx. 30% of HA[1)] and 5% of HB[2)] patients develop inhibitors making replacement therapy ineffective. Pharmacological doses of rFVIIa stop bleeding in inhibitor patients via bypassing FVIII and FIX in the coagulation cascade which requires FVIIa plasma activity significantly higher than physiological levels. However, the therapeutic window of FVIIa activity is unknown due to the complexity of relating pharmacokinetic (PK) /pharmacodynamic (PD) data, especially T½ and clearance, to efficacy and safety.
Methods: Limited rFVIIa data are available from PK studies in non-bleeding and bleeding haemophilia patients (HA/B), in patients with factor VII deficiency (FD) and in healthy subjects (HS). FVII activity has been assessed using two different assays: a FVII coagulation activity assay (detecting both endogenous FVII zymogen and FVII activity), and a FVIIa activity assay specific for activated FVII[3)].
Results: A comparison between the two assays has shown significantly different PK estimates and a simple conversion is not possible[3)]. The FVIIa activity assay is preferable for PK studies. PK parameters differed slightly but were statistically significant between bleeding and non-bleeding state (HA/B)[4)]. No effect of gender or ethnicity (HS)[5)] was seen whereas clearance was faster in children than adults[6,7)]. Dose proportionality was seen in HA/B[4,7)] and HS[5)]. FD patients have a higher clearance than HA/B[8)].
Conclusions: Haemophilia patients with inhibitors are few (~ 4000 in the Western world) and bleeding episodes are generally treated at home. Conducting PK studies in the bleeding state is therefore extremely difficult. Human subjects may be stratified in two groups based on rFVIIa PK – HS and adult HA/B vs HA/B children and FD. Relating PK profiles to clinical efficacy is complicated by few data and significant inter-patient and inter-bleed variations. Correlating PK parameters to efficacy and safety has so far not been possible.
1) Addiego Lancet 1993 2) Briet Blood Coagul Fibrinol 1991 3) Scharling Blood Coagul Fibrinol 2007 4) Lindley Clin Pharmacol Ther 1994 5) Fridberg Blood Coagul Fibrinol 2005 6) Hedner Haemophilia 1998 7) Villar Haemophilia 2004 8) Berrittini Haematologica 2001

Macromolecular Conjugates For Passive Tumor Targeting: *In Vitro* Studies With A Gelatin-Methotrexate Conjugate

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Background: Soluble macromolecular conjugates of low molecular weight anticancer agents are being investigated because of their potential to improve efficacy and reduce systemic toxicity based on their preferential accumulation in solid tumors.
Gelatin-methotrexate conjugates (G-MTX) have been investigated in our lab by evaluating the role of molecular weight, drug molar ratio, charge, and degradation on
growth inhibition of leukemia cells.
Methods: G-MTX prepared by carbodiimde reactions with different molecular weights of type B gelatin using SEC separations at each step produced molecular weights ranging from 35 to 182 kDa. Drug molar ratio determined by spectrophotometric methods ranged from 0.5:1 to 25:1 moles MTX : mole of gelatin. G-MTX charge measured by IEF electrophoresis ranged from 4.9 for anionic G-MTX to >9.6 for cationic G-MTX. Gelatin degradation of G-MTX by the lysosomal enzyme cathepsin B was evaluated after 90 min at the *in vitro* lysosomal pH of 4.8 using a trichloroacetic acid precipitation and BCA protein assay. Growth inhibition profiles of HL60 promyelocytic leukemia cells were conducted to obtain IC50 values.
Results: The IC50 values for anionic G-MTX with low drug molar ratio (0.5:1 to 2.2:1) ranged from 4.6x10-8 to 1.8x10-7 M and increased proportionately with molecular weight. The IC50 values for anionic G-MTX with high drug molar ratio (7.4:1 to 25:1) however, showed little if any molecular weight correlation, ranging from 2.0x10-7 to 2.9x10-7 M. IC50 values for cationic G-MTX of low drug molar ratio were about 4X higher than that for high drug molar ratio anionic G-MTX, except for the highest molecular weight specie which was about 10X higher at 2.9x10-6 M. The growth inhibition effect of MTX was reduced 5 to 290 times by conjugating to gelatin. The extent of G-MTX enzymatic degradation was 85% for the gelatin control and decreased from 67% to 30% as drug molar ratio increased from 2:1 to 23: 1.
Conclusions: 1) The inverse linear molecular weight effect with IC50 suggests the same mechanism for anionic G-MTX. 2) The non-linear molecular weight effect and substantially higher IC50 value for the highest molecular weight specie suggests a less effective and different mechanism for cationic G-MTX. 3) The apparent protection from degradation by high drug load may hinder intracellular drug release and efficacy compared to low drug load G-MTX.

Rectal Delivery of Ceftriaxone Sodium-loaded Mucoadhesive Gelatin-Mucin Microspheres

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Background: Biologically adhesive drug delivery systems such as microspheres offer important advantages over conventional drug delivery systems. The properties of bioadhesive microspheres such as their surface characteristics, force of bioadhesion, release pattern of incorporated drug and biodegradability are determined by the nature of the polymers employed in the formulation as well as their swelling and solvent regain characteristics. The reported interaction of mucin with many biologically important entities including polymers prompted the preparation of microspheres from admixtures of mucin and gelatin (a widely used pharmaceutical adjuvant). The objective of this study, therefore, was to prepare microspheres from admixtures of gelatin and mucin and to evaluate the rectal delivery of ceftriaxone sodium from these microspheres.
Methods: Microspheres based on mucin-gelatin admixtures (1:1, 1:2, 1:3, 1:4) and gelatin alone were prepared by the emulsifiction-crosslinking technique. Further experiments were carried out to evaluate the swelling and mucoadhesive characteristics of the formulated microspheres using water sorption in two media (SIF and SGF) and adhesion to the everted hog ileum as indices of measurements respectively. Drug loading was achieved by diffusion-filling at 37 ± 0.2 ^{o}C using a concentrated solution of ceftriaxone sodium in citrate/phosphate buffer (pH 7.4) and finally determining the drug content of a given quantity of the loaded microspheres spectrophotometrically at 240 nm. Release studies were carried out in simulated gastric fluid without pepsin (pH 1.2) and simulated intestinal fluid without pancreatin (pH 7.4) to evaluate the release behavior of the microspheres. For the rectal delivery studies, three groups each of eight Male Wistar rats were used. An amount of the microspheres containing ceftriaxone sodium was weighed out to achieve a dose level of 100 mg/kg body weight in the rats and carefully transferred into empty hard gelatin capsule (No. 3). A positive control was set up by similarly encapsulating amounts of pure ceftriaxone sodium equivalent to that in the microspheres. At regular time intervals of 1 h, 0.5 ml of blood was sampled from the orbital sinus of the rats and then analysed for plasma levels of ceftriaxone sodium using the method of Tietz[5] to prepare a protein-free filtrate prior to spectrophotometric analysis.
Results: The formulated microspheres showed good mucoadhesive properties and exhibited percentage mucoadhesion as high as 80 % in some cases. Mucoadhesion to hog everted intestinal tissue was found to be comparatively higher in SGF than in SIF. Preliminary liquid uptake studies showed that water absorption and the rate of water absorption by the microspheres followed the order:1:4>1:1>1:2>1:3>gelatin alone in SGF while the order in SIF is 1:4>gelatin alone>1:3>1:1>1:2 (data not shown). The release profiles of ceftriaxone sodium from the mucoadhesive microspheres in two different release media (SIF and SGF) show high percentage and rapid release of ceftriaxone from the microspheres in SIF within 30 min. The plasma level-time curve for the rectally administered ceftriaxone loaded mucoadhesive microspheres is shown in Fig. 1 The areas under the plasma concentration versus time curves (AUC) were determined using the trapezoidal rule based on a non-compartmental pharmacokineticanalysis. The pharmacokinetic parameters as calculated from Fig. 1 show that the bioavailability of ceftriaxone sodium via the rectal route was highest (AUC = 444.8 μg-h ml^{-1}) from microspheres prepared from equal portions of S-mucin and gelatin (1:1). Microspheres prepared from gelatin alone gave significantly lower (P≤ 0.05) AUC value in comparison with those prepared from its admixtures with S-mucin. The generally high AUC values obtained for the microspheres may suggest that the absorption of ceftriaxone sodium from the rectal mucosa was both rapid and complete and that the drug must have by-passed the hepatic first pass metabolism. It is necessary to point out that adequate precautions were taken to deposit the encapsulated microspheres on the lower part of the rat's rectum with the expectation of causing the absorbed drug to drain directly into the general circulation via either the lower or middle haemorrhoidal veins. This target may well have been achieved considering the high AUC values obtained for all the microspheres.
Conclusion: 1) The microspheres showed considerably high mucoadhesive capacities with good swelling profiles. 2) The rectal route could provide a therapeutically viable alternative for the delivery of ceftriaxone sodium, which hitherto, is administered only parenterally owing to its instability in the acidic environment of the stomach.

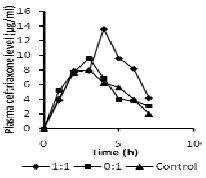

Fig. 3: Plasma ceftriaxone sodium versus time profiles for the microspheres

Development of Gelatin-Based Nanoparticulate Formulation for the Delivery of Human Insulin

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Background: The design of systems for oral delivery of proteins is in fact really challenging and comparatively very little work has been reported in this field of research. However, the tendency of proteins to denaturation and loss of bioactivity poses severe limits to the reactions that can be performed on the carrier, to the solvents that can be used, and to the environmental conditions that can be adopted during preparation, purification, and storage of the delivery system. Given the above background, we developed a nanoparticulate formulation based on gelatin for the delivery of human insulin. In this study, we report the first successful encapsulation of insulin into gelatin nanoparticles (NPs) under very gentle nanoprecipitation conditions.
Methods: Unloaded and insulin-loaded gelatin (type B, 225 bloom) NPs were prepared by a one-step desolvation technique at a pH of 7.0. and characterized. To determine the amount of incorporated and free insulin, the NPs were recovered by evaporating the solvent using a Rotavapor at 30 ^{o}C under reduced pressure until about 15 ml of the dispersion remained. The recovered dispersion was centrifuged at 14,000 × g for 20 min and the supernatant was assayed for insulin by reverse phase high performance liquid chromatography (RP-HPLC) to determine the amount of free insulin. The sediment was redispersed in 10 ml of 0.01 N HCl and shaken for 10 min to dissolve any insulin adhering to the surface of the NPs and further subjected to centrifugation. The resulting supernatant was analysed to determine the amount of insulin entrapped on the surface of the NPs. The last sediment was finally digested in 5 ml of trypsin solution (0.4 mg/ml) to release all insulin entrapped within the matrices of the particles. The insulin content of the solution was immediately determined. The in vitro release of insulin from the NPs was performed in 10 ml each of simulated gastric fluid (pH 1.2) and in phosphate buffered saline (pH 6.8) without enzymes.
Results: The most challenging aspect of this study was the search for appropriate cross-linking agent needed to stabilize the NPs. Glutaraldehyde (GTA) has been employed routinely by most investigators to cross-link gelatin-based NPs. However, in this study an instantaneous loss of insulin monomers was observed upon addition of glutaraldehyde to the NPs in situ. This was evident from the disappearance of the retention peak (7.7 min) of insulin earlier recorded in a RP-HPLC chromatogram for pure human insulin (Fig.1). Evaluation of alternative cross-linking agents (formaldehyde and glyoxal) led to the selection of glyoxal as an effective cross-linker for gelatin NPs.

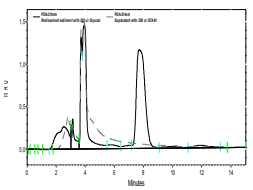

Fig. 1:RP-HPLC chromatogram of insulin after entrapment into gelatin NPs cross-linked with GTA (……) and with glyoxal ( )

Glyoxal did not cause any loss of monomeric insulin (Fig. 1). The optimum amount of glyoxal needed to effectively cross-link the NPs was found to be 120 mg per 200 mg of gelatin while amounts of glyoxal in excess of 200 mg (500 µl) led to gradual aggregation and precipitation of the particles with the rate and intensity of aggregation increasing with an increase in the amount of glyoxal added. Mean size of between 500 and 600 nm monodisperse NPs were obtained. Unloaded and insulin-loaded NPs were found to have zeta potential values of -20.1 and -16.8 mV respectively. The amount of insulin loaded on the surface of the NPs (200 mg) was evaluated to be 13.67 mg which represented 83.6 % of the total amont of drug used in the loading studies. The amount of unentrapped insulin was 8.84 % while 7.6 % was entrapped into the matrices of the NPs. The release profile of insulin from the optimized nanoparticulate formulation shows that in simulated gastric medium, a burst release occurred within the first 30 min possibly due to the rapid leaching off of the surface bound insulin and about 55 % of the entrapped drug was released over a 6 h release period. In simulated intestinal medium (pH 6.8), however, release of the entrapped drug was slow and sustained after the initial burst release and up to 80 % of the entrapped insulin was released over 6 h.

CONCLUSION: 1) Stabilized insulin-loaded gelatin NPs could be formulated under gentle nanoprecipitation conditions using optimal amounts of glyoxal as the cross-linking agent in order to preserve the chemical stability of insulin. 2) Release of insulin depended greatly on the stability of the NPs at distinct pH environments.

Screening Of Antioxidants For Inhibitory Activity Against Lung Metastasis Of Murine Colon Cancer Cells In Mice

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Background: Cancer metastasis is a major cause of death in cancer patients. Thus, it is important to find promising agents with antimetastatic activity. A variety of antioxidants such as antioxidative vitamins and polyphenols have recently been proposed to be effective in inhibiting cancer metastasis mainly based on the inhibition of tumor invasion, growth and angiogenesis. However, it is unclear which antioxidant has more potent antimetastatic activity. Here, a comparative study on the inhibitory activity of 20 antioxidants against the *in vitro* invasion, growth and experimental lung metastasis of murine colon cancer cells was conducted, and a promising agent was further examined on anti-tumor immune cells.
Methods: Experimental lung metastasis assay was performed by inoculation of murine colon 26-L5 cells through a tail vein of mice and tumor nodules on the suface of lungs were determined. Test compounds were administered intraperitoneally 5 consecutive times beginning 3 days before tumor inoculation at a dose of 1 micromol. Cell invasion and growth were evaluated by transwell chambers and WST-1 solution, respectively. Free radical scavenging assay was carried out using the stable radical, 1,1-diphenyl-2-picrylhydrazyl. In some experiments, mice receiving anti-asialoGM1 or 2-chloroadenosine, or nude mice were used to examine roles of natural killer (NK) cells, macrophages, or T cells, respectively, in the antimetastatic effect of a test compound.
Results: Among 20 compounds tested, epigallocatechin gallate (EGCG) exhibited most significant reduction by 77% in tumor metastasis. EGCG also inhibited it dose-dependently with 98% suppression at 2 micromol Statistically significant relation was not observed between the radical scavenging, tumor invasion and growth inhibiting activities of test compounds and their inhibition rates of tumor metastasis. We have next examined roles of anti-tumor immunity in the EGCG's effect, and found that depletion of NK cells completely abrogated the effect, whereas a marginal reduction of the effect was seen in nude mice and macrophage-depleted mice.
Conclusions: These results suggest that EGCG has potential benefit for tumor metastasis inhibition. Its antimetastatic effect may be mediated mainly through NK cell activity.

Radiotherapy And Multi-Agent Chemotherapy (Procarbazine, ACNU And Vincristine) For High-Grade Gliomas: A Prospective Study

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Purpose: To prospectively evaluate the efficacy and toxicity of radiotherapy combined with multi-agent chemotherapy using procarbazine, nimustine (ACNU) and vincristine in adults with high-grade gliomas.
Materials and Methods: Patients aged 18 years of age or older with a histologically confirmed supratentorial glioblastoma or grade 3 gliomas (anaplastic astrocytoma, anaplastic oligoastrocytoma and anaplastic oligodendroglioma) were enrolled. Radiotherapy protocols were hyperfracionated radiotherapy (HF-RT) or radiotherapy immediately after hyperbaric oxygenation (HBO) (HBO-RT). The protocol of HF-RT consisted of 2 fractions per day of 1.2 Gy to a total dose of 72 Gy (10 fractions per week). The protocol of HBO-RT consisted of daily 2 Gy fractions for 5 consecutive days per week up to a total dose of 60 Gy, with each fraction administered immediately after HBO with the period of time from completion of decompression to irradiation being less than 15 minutes. Chemotherapy consisted of procarbazine (90 mg/m^2 orally, days 1 to 14), ACNU (80 mg/m^2 intravenously, day 1) and vincristine (0.5 mg/m^2 intravenously, days 1 and 8) and was administered during and after radiotherapy, up to a maximum total of 4 courses.
Results: Between 1997 and 2003, a total of 51 patients (36 patients with glioblastoma and 15 patients with grade 3 gliomas) were enrolled. HF-RT was administered in 10 patients treated between 1997 and 1999, and HBO-RT was administered in 41 patients treated between 2000 and 2003. All 51 patients were able to complete a planned radiotherapy using HF-RT or HBO-RT with 1 course of concurrent chemotherapy. Of 38 assessable patients, 20 (52%) had an objective response including 6 CR and 14 PR. The median survival time in all 51 patients and 36 glioblastoma patients were 17.5 months and 15.5 months, respectively. On univariate analysis, histologic grade (p < 0.0001) and Karnofsky performance status (p = 0.01) had a significant impact on survival, and on multivariate analysis, histologic grade alone was a significant prognostic factor for survival (p < 0.001). Although grade 4 leukopenia and grade 4 thrombocytopenia occurred in 10% and 8% of all patients respectively, these were transient with no patients developing neutropenic fever or intracranial hemorrhage. No serious non-hematological or late toxicities were seen.
Conclusions: These results indicated that radiotherapy with multi-agent chemotherapy (procarbazine, ACNU and vincristine) was effective and safe with virtually no late toxicity in patients with high-grade gliomas

Exploiting Biochemical Differences Between Parasite And Host In Development Of Antiparasitic Agent

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Background: Protozoans are auxotrophic for purine bases and nucleosides and hence depend on exogenous sources to meet their purine requirements. Differences between the nucleoside carrier systems of host cells and the parasites could be exploited in designing rationale chemotherapy involving nucleoside transport inhibitor (NTI) like (6-[4-nitrobenzyl)-mercapto]purine ribonucleoside (nitrobenzylthioinosine; NBMPR) and toxic nucleoside analogue compounds. Aim: 1) To determine number of NBMPR binding sites on animal cells and trypanosome trypomastigotes. 2) To determine whether co-administration of NBMPR and toxic nucleoside analogue, tubercidin, could reduce parasitaemia in animal infected with *Trypanosome brucei gambiense* and hence prolong the survival of the infected animal.
Methods: Measurement of number of NTIs in animal cells was done using erythrocytes from human, mouse and hamster. NBMPR binding parameters (K_d and B_{max}) were determined from Scatchard plots using appropriate computer program (Elsevier-Biosoft Dose-effect analysis) performing a straight fit (B/F vs B) to data sets. In assay to determine survival and parasites clearance, two groups of mice (5 mice/group) were inoculated with 0.2 ml of the parasite suspension containing 10^6 trypanosomes/ml. When parasitaemia had reached 10^5 trypomastigotes/ml blood, the animals received single doses of tubercidin (10.5mg/kg) or tubercidin in combination with NBMPR (5mg/kg). Tail blood was examined daily for parasites for 7days and survival of animal followed for 30 days.
Results: Binding parameters are shown in the table below:

Species	K_d (nM)	B_{max} (sites/cell)
Human	1.86±0.41	3531±409
Mouse	2.77±0.27	8821±612
Hamster	1.02±0.41	674±132

Values are means±SE, N=3
Trypanosome trypomastigotes did not have measurable NTI binding sites.
Mice that received tubercidin-NBMPR combination were cleared of parasites and survived beyond the observation period while all that received tubercidin singly died.
Conclusions: The NTI which protected the host cell against tubercidin toxicity did not protect the parasites thus leading to parasite clearance while the group that received only tubercidin died from the effects of tubercidin toxicity.

A Novel Polymorphic Purine Complex At The 1.5 kb Upstream Region Of The Human Caveolin-1 Gene And Risk Of Alzheimer?s Disease; Extra-Short Alleles And Accumulated Allele Homozygosity

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Crucial interaction of caveolin-1 (CAV1) with b- and g-secretases, and aberrant expression of the gene encoding this protein in Alzheimer?s disease (AD) support a role for CAV1 in the pathophysiology of this disease.
We report a novel polymorphic purine complex stretching ~150 bp of genomic DNA at the 1.5 kb upstream region of the human CAV1 gene, alleles and genotypes of which are associated with sporadic late-onset AD. Extra-short alleles were observed in the case group that were absent in the control subjects. Increased homozygosity for haplotypes was also observed at this region in the Alzheimer?s cases (p<0.002). This region contains GGAA and GAAA motifs, the consensus binding sites for the Ets and IRF family transcription factors, respectively, and is highly conserved in distantly-related non-human primates in respect with location and motif sequence. The effect of this complex sequence on the expression of CAV1, and the related mechanisms in the pathophysiology of AD remain to be clarified.

Transient Drugs As Magic Bullets: A New Approach To Drug Discovery

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A plethora of weak/transient biological interactions (dissociation constant: K_D > μM), either working alone or in concert, occurs frequently throughout biological systems. We are beginning to realize their importance in complex biological networks for the support of life. This appreciation has important implications in for example drug discovery as we can question the current paradigm of drug design to find the highest possible binders (drugs) to a given target (receptor). Development of transient drugs as magic bullets, defined by their weak binding to target, can be based on high-off-rates, multivalent approaches or multiple targets. Now, high throughput techniques are available to discover such drug candidates and diverse molecular libraries are available. The greatest problem yet to overcome is maybe the mind-set of the individual researcher that weak/transient binders are undesired and therefore of no benefit.
I will introduce you to the basic concepts of transient binding and their role in biological systems, define and discuss benefits of a transient drug, show potential screening procedures to find them, speculate about possible targets for transient drugs including cardiovascular diseases, CNS disorders and pain conditions.

Development Of Highly Anti-HIV Active And Lowly Toxic 4'-*C*-Ethynyl-2'-Deoxy-2-Fluoroadenosine And A Proposal Of The Way To Develop Highly Active And Lowly Toxic Antiviral Modified Nucleosides

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Background: The existing antiretroviral therapy has several critical problems such as (i) the emergence of new drug-resistant HIV-1 mutants, (ii) the need to take large dosages of drugs, and (iii) drug side effects.
In order to solve these problems three working hypotheses, (i) the way to prevent the emergence of resistant HIV-1 mutants against nucleoside reverse transcriptase inhibitors, (ii) the way to decrease the toxicity of nuleosides, and (iii) the way to provide nucleosides with stability to both enzymatic and acidic glycolysis, were proposed in our study.
Methods: Based on the working hypotheses, many kinds of 4'-*C*-substituted nucleosides were synthesized and evaluated for their biological activity and stability to both enzymatic catabolism and acidic degradation.
Results: The scientific evidences obtained by our study proved the validity of the three working hypotheses and resulted in the development of 4'-*C*-ethynyl-2'-deoxy-2-fluoroadenosine(two positions modified 2'-deoxyadenosine derivative), which is highly potent against all HIV-1s, does not have acute mouse toxicity, is stable to intracellular enzymatic catabolism and acidic degradation, its triphosphate has a very long intracellular half-life, does not greatly inhibit mitochondria DNA polymerase γ.
Conclusions: 1) 4'-*C*-ethynyl-2'-deoxy-2-fluoroadenosine which is highly active against all HIV-1s and lowly toxic was developed. 2) The proposed three working hypotheses were proved to be valid. 3) The working hypotheses could be generally applied to the development of new highly active and lowly toxic antiviral modified nucleosides.
Authors' disclosure statement:
Merck's scientists developed a highly anti-HCV active and lowly toxic 2'-*C*-methyl-7-fluorotubercidine which is a two positions modified adenosine derivative.
Our study and Merck's study showed that human DNA and RNA polymerases are more sophisticated than viral polymerases in the point that they scarcely accept the nucleosides modified at two or more positions of physiologic nucleosides into their active centers but viral polymerases do accept them, and further suggested that by taking advantage of the difference it will be possible to develop highly active and lowly toxic antivital modifie nucleosides, especially those against Flu-virus because Flu-virus uses RNA-dependent RNA polymerase like as HCV does.

Blockade Of IL-6 Signalling With A Humanized Anti-IL-6 Receptor Antibody, Tocilizumab, For The Treatment Of Rheumatoid Arthritis

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Background: IL-6 is a proinflammatory cytokine and is known to play important roles in the pathogenesis of rheumatoid arthritis (RA). Several clinical studies have demonstrated that blockade of IL-6 signalling by a humanized anti-IL-6 receptor antibody is highly effective in the treatment of patients with active RA who are inadequately responsive to traditional anti-rheumatic drugs. IL-6 has a variety of biological activities that match many of the symptoms observed in RA patients. The precise mechanisms of action of tocilizumab are still not fully understood, however.
Methods: We reviewed possible mechanisms of action of tocilizumab, based on recently published papers.
Results: The data from Phase III clinical studies confirmed that tocilizumab can improve symptoms (including the number of swollen joints, the number of tender joints, fever, fatigue, anaemia and anorexia) in moderate to severe active RA. It has also been reported that tocilizumab prevented the radiographic progression of joint destruction. Tocilizumab has generally been well tolerated. Interestingly and importantly, serum IL-6 levels gradually decreased during long-term treatment, even though tocilizumab does not directly inhibit the synthesis of IL-6. This might be explained by the finding, in an animal model, that blockade of IL-6 signalling suppressed the induction of Th17 cells, which play a pathogenic role in the development of autoimmune diseases. In addition, we recently found that tocilizumab inhibited IL-6-induced RANK ligand expression on synovial cells obtained from RA patients, resulting in the inhibition of osteoclast formation. Tocilizumab also inhibited the gene expression of vascular endothelial growth factor, which causes neovascularisation that increases the supply of oxygen and nutrition to growing synovial tissues.
Conclusion: Clinical studies have demonstrated that targeting the IL-6 signalling pathway with tocilizumab could be an attractive and innovative therapeutic option for RA. It is highlighted that high efficacy was achieved consistently in several studies, and this adds to the evidence for the deep involvement of IL-6 in the pathogenesis of RA.
In addition, blockade of IL-6 signalling inhibited the induction of Th17 cells and inhibited angiogenesis and bone destruction.

Reconstruction Of *In Vivo* P-Glycoprotein Activity From *In Vitro* Information By Targeted Absolute Proteomics

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Background: The reconstruction of P-glycoprotein (P-gp) function at human blood-brain barrier (BBB) from *in vitro* studies is a key issue for pharmaceutical industries, because P-gp plays a significant role to limit drug penetration across the BBB, influencing directly to pharmacological effect and toxicity in brain. The purpose of the present study was to reconstruct *in vivo* function of P-gp at BBB based on its absolute expression amount and *in vitro* transport activity in mouse.
Methods: *In vitro* P-gp transport activity was determined by the transcellular transport study using the monolayer of mouse P-gp transfected LLC-PK1 cells (L-mdr1a) and parental LLC-PK1 cells, which were kindly provided by Dr. Schinkel. P-gp absolute expression amount was determined according to the targeted absolute proteomics we developed (Kamiie et al., *Pharm Res,* 2008). Brain-to-plasma concentration ratio ($K_{p\ brain}$) was measured by intravenous constant infusion, and $K_{p\ brain}$ ratio was calculated by dividing the $K_{p\ brain}$ in P-gp knockout mice by that in wild-type mice.
Results: As a parameter of *in vivo* function of P-gp at mouse BBB, $K_{p\ brain}$ ratio was reconstructed. First, intrinsic transport activities per P-gp molecule for 6 substrates were determined by dividing the *in vitro* P-gp transport activities by absolute expression amount of P-gp protein in L-mdr1a (15 fmol/μg protein). Then, by combining the obtained intrinsic transport activities with absolute expression amount of P-gp protein in mouse brain capillaries (14 fmol/μg protein), the $K_{p\ brain}$ ratio was reconstructed (quinidine, 31; loperamide, 23; digoxin, 17; dexamethasone, 11; vinblastine, 12; cimetidine, 3.6). The reconstructed $K_{p\ brain}$ ratio from *in vitro* studies corresponded well with the observed $K_{p\ brain}$ ratio determined in mouse *in vivo* study (quinidine, 39; loperamide, 31; digoxin, 20; dexamethasone, 8.8; vinblastine, 8.0; cimetidine, 1.4) for all 6 substrates.
Conclusions: The present study demonstrated in mouse that absolute values of $K_{p\ brain}$ ratio can be reconstructed by integrating P-gp absolute expression amount and in vitro P-gp transport activity. Applying this approach to human, it is possible to reconstruct P-gp function at human BBB from in vitro transport studies by using quantitative human transporter atlas of the BBB.

Drug Screening Of Preserved Oral Fluid By Liquid Chromatography-Tandem Mass Spectrometry

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Background: Oral fluid is an alternative matrix for drug analysis with potential applications in road-side drug screening, work-place drug testing, drug treatment programs, and epidemiological surveys.
Methods: We have developed a liquid chromatography–tandem mass spectrometry (LC-MS/MS) method for drug screening of preserved oral fluid, collected with either Intercept® (Øiestad *et al. Clin. Chem.* **53** (2) (2007), pp. 300–309).or Saliva Sampler® collection devices. Samples were prepared by liquid–liquid extraction with ethylacetate/heptane (4:1). LC-separation was achieved with an Atlantis dC18-column (2.1 X 50 mm, 3 μm particle). Mass detection was performed by positive ion mode electrospray LC-MS/MS and included the following drugs/metabolites: morphine, 6-monoacetylmorphine, codeine, buprenorphine, methadone, amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxyethylamphetamine, cocaine, benzoylecgonine, Δ-9-tetrahydrocannabinol, lysergic acid diethylamide, alprazolam, bromazepam, clonazepam, 7-aminoclonazepam, diazepam, N-desmethyldiazepam, 3-OH-diazepam, fenazepam, flunitrazepam, 7-aminoflunitrazepam, lorazepam, nitrazepam, 7-aminonitrazepam, oxazepam, zopiclone, zolpidem, carisoprodol, and meprobamat. The method has been used to analyse approx. 10800 samples from drivers in Norwegian traffic stopped at random (Gjerde *et al*, Accid. Anal. Prev., in press http://dx.doi.org/10.1016/j.aap.2008.06.015) and is currently being used for the DRUID project (http://www.druid-project.eu). Application of the method for drug screening in an opioid maintenance treatment program is currently under evaluation.
Results: Screening of 28-32 drugs was performed with a run time of 14 min. Within- and between-day relative CVs with the Intercept device varied from 2.0% to 31.8% and from 3.6% to 39.1%, respectively. Extraction recoveries were >50% except for morphine (30%) and benzoylecgonine (0.2%). Experiences with application of the method and differences in matrix effects and absorption to the sampling device for the two devices will be discussed.
Conclusions: The method allow rapid and sensitive oral fluid screening for the most commonly abused drugs in Norway and have been successfully applied to a large number of samples.

Taxane-Based Tumor-Targeting Anticancer Agents

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Background: A long-standing problem in cancer chemotherapy is the lack of tumor-specific treatments. Therefore, the development of innovative and efficacious tumor specific drug delivery protocols or systems is urgently needed. Rapidly growing tumor cells overexpress many tumor-specific receptors, which can be used as targets to deliver cytotoxic agents into tumors.
Methods: We designed and developed new taxane-based anticancer agents possessing tumor-targeting ability and efficacy against various cancer types, especially drug-resistant tumors. These new anticancer agents are conjugates of the second-generation taxane anticancer agents with tumor-targeting modules through mechanism-based self-immolative disulfide linkers, which are specifically delivered to tumors, internalized into tumor cells, and the potent anticancer agents are released from the linker into the cytoplasm.
Results: We used monoclonal antibodies (for EGFR) and omega-3 polyunsaturated fatty acids, in particular DHA, as tumor-targeting molecules for drug conjugates, which exhibited remarkable efficacy against human tumor xenografts (e.g., A431, A121, DLD-1, CFPAC-1) in mouse models. Vitamin receptors are excellent biomarkers for cancers. Thus, biotin and folate were successfully employed as tumor-targeting molecules as well. In order to monitor and elucidate the mechanism of tumor-targeting, internalization and drug release, several fluorescent and fluorogenic probes were developed The use of functionalized single-wall carbon nanotube (SWNT) as a vehicle for drug conjugates bearing multiple guiding modules and warheads led to the development of novel nanopharmaceutical agents.
Conclusions: 1) DHA-SB-T-1214 provided highly promising results which identified this drug conjugate as the leading candidate for drug development. 2) We succeeded in monitoring the receptor-mediated endocytosis, drug release, and drug-binding to the target protein, microtubules, by means of confocal fluorescence microscopy. 3) We found that the mass drug delivery into the cytosol of the cancer cells using our novel drug delivery system (DDS) is superior than the simple exposure of the drug itself to the same cancer cells. These results strongly suggest that the functionalized SWNT-based DDS can serve as a highly promising drug delivery platform.

Efficient Cancer Therapy By Use Of Neovascular-Targeted Liposomes

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Background: Since tumor angiogenesis is critical for tumor growth and blood borne metastasis, antiangiogenic therapy has been focused on for the tumor treatment. Furthermore, damaging angiogenic vessels would eradicate tumor due to cutoff the nutrients and oxygen. For this purpose we isolated a novel neovessel –targeted peptide, Ala-Pro-Arg-Pro-Gly (APRPG), from a phage displayed library, and liposomes were modified with APRPG-conjugate of distearoylphosphatidyl ethanolamine-polyethylene glycol (PEG-DSPE) for the specific delivery of antiangiogenic or anticancer agents to angiogenic endothelial cells.
Methods: In vivo distribution of radio-labeled APRPG-PEG-DSPE-modified liposomes (APRPG-PEG-Lip) and intratumoral distributions of APRPG-PEG-fluorescence-labeled APRPG-PEG-Lip were determined with radioactivity and LSM, respectively. Tumor growth suppression by the treatment of SU1498, an angiogenic inhibitor, or doxorubicin (DOX) encapsulated in APRPG-PEG-Lip was examined.
Results: APRPG-PEG-Lip accumulated in tumor similarly to control PEG-Lip, while intratumoral distribution was quite different: The former co-localized with angiogenic vessels and the latter accumulated interstitial tissues of the tumor around the vessels. SU1498 encapsulated in APRPG-PEG-Lip significantly suppressed tumor growth suggesting APRPG-PEG-Lip could deliver the antiangiogenic agent effectively to angiogenic endothelium. Moreover, DOX-encapsulated APRPG-PEG-Lip strongly induced apoptosis of tumor cells and suppressed tumor growth in tumor-bearing mice. This suggests that the damaging angiogenic endothelium by anticancer agents causes indirect and efficient eradication of tumor cells.
Conclusions: Tumor angiogenic endothelial cells are easily targeted by magic bullet, since the active-targeting drug carriers primarily interact with the cells after injection into bloodstream. Therefore, neovascular-targeted liposomes effectively deliver antiangiogenic or anticancer agents to neovessels and efficiently suppressed angiogenesis or damaged anigogenic vessels, respectively. Neovascular-targeted liposomes would be useful DDS carriers for the cancer treatment.

Is The Induction Chemotherapy Response- And Recurrence Rate Depend On N0 Or N+ Stage In Oral Squamous Cell Cancer?

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Background: In Hungary the morbidity and mortality rate of head-neck cancer have increased in the last 30 years. One of the most important treatment failure is the high rate of primary recurrences after surgery. In this study the authors compare the regression and recurrence of N0 and N+ cases following chemotherapy.
Methods: During a 6 year period, 90 consecutive treated OSCC patients were entered into this retrospective study. From these patients 39 were N0 and 51 were N+ stages. Twenty-five patients received Bleomycin- Vincristrin- Methotrexate (BVM), twenty BVM+ Cisplatine and forty-five BVM+Mitolactol neoadjuvant chemotherapy. After three courses of chemotherapy the regression (CR,PR,NR) and side effect rate were determined. All of the patients were operated and observed the number and localisation of recurrences in three years follow-up time.
Results: The N0 cases came from T2-3, while N+ from T2-4. The regression were in N0 group CR:46%, PR:54%, but in N+ group CR:12%, PR:74%, NR:14%. The side effects were slight (grade I-II) and reversible. The recurrence rate for N0 group was 15%(lymph node metastasis), while for N+ group 59% (primary or metastasis). The recurrence rate was only 4% for complete responders. The three year tumor-free survival for N0 group 85%, N+ group 43%.
Conclusions: After neoadjuvant chemotherapy there was very good chance for tumor free survival in complete- coming from N0-N+ stages and partial responders coming from N0 stage. For these cases (45 patients) the recurrence rate was 13%. The chance was bad for partial- or no-responders coming from N+ stage (45 patients) as the recurrence rate was 70%.

High Uptake Of Vaccines – A „Magic Bullet" In Control The Burden Of Hospitalisation Attributable To Childhood Mumps And Rotavirus Infections

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Background: The Polish immunisation program consists of two vaccination systems; the routine (obligatory) and recommended (optional) vaccination. The routine vaccinations (mumps) are bought by Polish National Health Fund and recommended vaccinations (rotavirus [RV]) are paid by individuals. Aim: To assess the impact of different immunisation systems on the hospitalisation burden attributable to vaccine-preventable mumps and RV infections in children between 2003 and 2008, in north-eastern Poland.
Methods: A retrospective analysis of hospital discharge data obtained between January 9, 2003 and June 30, 2008, at University Children's Hospital in Bialystok. Electronic hospital discharge data were reviewed to select records of children 0-18 years of age with ICD-10 codes for RV gastroenteritis (RVGE – A08.0) and for mumps (B26.0; B26.1; B26.3; B26.9). The nosocomial RV infections in 2008 were analysed based on the Hospital Infection Control Team database. The results were compared with mumps vaccines uptake (web database of the National Institute of Hygiene in Warsaw) and local RV vaccines uptake.
Results: Of 5213 children hospitalised during study period 2003-2007, 626 (12%) were discharged as RVGE, and 319 (6.1%) as mumps infection. The proportion of hospitalisations attributable to the mumps infection declined from 15.9% in 2003 to 0.2% in 2007 year, in line with increasing mumps vaccination uptake. In the year 2003, 39% of Polish children have been vaccinated against mumps whereas in 2007 – 97%. In the first 6 months of 2008 no case of mumps was hospitalised. The percentages of hospitalised cases attributable to RVGE increased during the study period from 7.7% in 2003 to 18.1% in 2007. In the first half of 2008, the percentage of hospitalised RVGE cases reached the highest level (39.7%). In 2008 year, the percentage of nosocomial RV cases among total RV cases was estimated to be 16.1%. In 2007, 89 infants under 6 months of age out of 1997 children born in our region were vaccinated against RV (vaccine coverage – 3%).
Conclusion: Routine vaccination system in line with high uptake of RV vaccines are needed to provide herd immunity and to control the burden of hospitalisation.

Treatment Outcome Of Otomycosis With 1% Clotrimazole Cream

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Among 141 patients suspected of having otomycosis, 76(53.9%) were mycologically confirmed. The fungi isolated were Aspergillus sp (63.4%), Candida (35.5%) and Mucor
(1.3%). Ninety-six per cent were symptom free within 2 weeks of topical application of 1% clotrimazole cream, after thorough cleaning of debri in the ear canal. Treatment failures were minimal, including recurrence (2.6%), 'acute otitis externa (1.3%), foreign body in the ear (1.3%) and blocking of ear by therapeutic agent (2.6%).
This treatment regimen is simple, efficacious, cost effective and safe; hence it is recommended for adoption in the management of otomycosis.

B16 And Cloudman S91 Mouse Melanoma Cells Susceptibility To Apoptosis After Dacarbazine And Doxorubicin Treatment Examined In Three Cytotoxicity Tests: Cell Counting, MTT And Flow Cytometry

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Background: Considering the necessity of an individual choice of cytostatic drugs for patients with cancer disease and tumor cells' resistance to these compounds, their ability to induction of apoptosis should be investigated. The aim of this study was to determine the influence of dacarbazine (DTIC) and doxorubicin on morphology and kinetics of proliferation of B16 and Cloudman S91 cells.
Methods: CELL CULTURE: Two rodent melanoma cell lines were used in cytotoxicity tests. Melanoma cells of both lines differ in the degree of pigmentation and in the growth rate in vivo. Cell were cultured in the Dulbecco medium with the addition of glucose, fetal bovine serum, penicilin, streptomycin, gentamycin and diflucan and harvested using 0.04% EDTA in PBS devoid of ions Ca^{+2} and Mg^{+2}.
CYTOTOXICITY STUDIES: Dacarbazine was used at concentrations 0.001-3.568M and doxorubicin at concentrations 0.0002 – 0.0431M.*Cell counting* – melanoma cell were cultured in 12 well plates 24h and then incubated with tested drug in order of decreasing concentration. After 24 h drug solutions was removed and the fraction of viable cells was a pellet. Fixed by washing with 70% ethyl alcohol cells were dried and counted. *MTT test* was carried out in a 96-well round bottom microtitre plates. *Apoptotic and necrotic assay by flow cytometry:* B16 and Cloudman S91 cells were stained with annexin V-FITC and propidium iodide and analyzed by the flow cytometer EPICS XL (Coulter).
Results*: Cell morphology studies:* After treating with dacarbazine (1.098M) B16 cells were converted to the oval shape. Characteristic for melanomas in culture spindle-like cells were absent in both of examined cell lines. Cellular atypia of B16 cells was characterized by abnormal structure of nuclei (giant) and hyperchromatosis. Doxorubicin has changed the morphology of both cell lines too. They became dendritic-like cells with long branches. Irregularity of nuclei was also observed.
Cells viability after exposure to cytostatic drugs were established by three methods and the assesing value EC50 being compared.methods of direct counting of alive cells and colorimetric methods assesing cell viability are characterized by a relatively low accuracy. The highest percentage of B16 and Cloudman S91 cells resistant to cytostatic drugs was received by flow cytometry and this method is recommended as the most accurate for drug cytotoxicity evaluation. Cloudman S91 cells were more resistant to dacarbazine (EC50 = 2.740M) and doxorubicin (EC50 = 0.0040M) than B16 (respectively EC50 = 1.644M and EC50 = 0.0024M). Maximum percentage of apoptotic B16 cells after DTIC exposure (11%) was lower than Cloudman S91 cells (22.2%). B16 cells underwent apoptosis after exposure to lower doxorubicin concentration than Cloudman S91. Maximum percentage of apoptotic B16 cells after doxorubicin exposure (64.7%) was higher than Cloudman S91 cells (59%). DTIC induces cell arrest in the G2/M and S cell cycle phase, doxorubicin arrested both investigated cell lines at phase S.
Conclusions: In our work, it was shown that doxorubicin is radical-generating agent, induced cell death in B16 and Cloudman S91 cells via apoptosis pathway. Our results may suggest that the inefficient therapy of human malignant melanoma can be connected to very low apoptotic cells number after DTIC treatment.

Nicotine Dramatically Increases Impulsive Behavior–Can Nicotinic Acethylcholine Receptor Antagonist Suppress Impulsive Behavior?

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Background: Impulsivity has been associated with drug addiction, aggression, criminal involvement, and suicide. Impulsive behavior is becoming a social problem in many countries. Recently, the relationship between nicotine and impulsive behavior is attracting attention. Aims: 1) To determine the relationship between the estimated dose of nicotine intake per day and impulsive behavior in human. 2) To examine the acute effect of nicotine on impulsive behavior in rats. 3) To examine whether nicotinic acethylcholine receptor (nAChR) antagonist could suppress impulsive behavior in rats.
Methods: Twenty seven habitual smokers and 23 never smokers between 21 and 33 years of age participated in human study. In animal studies, subjects were male Wistar-strain rats (10-13 weeks old). In human study, delay and probability discounting task was employed to assess impulsivity. In animal studies, 3-choice serial reaction time task was used to assess impulsivity and the effects of nicotine (0, 0.05, 0.1, 0.2, 0.4 mg/kg, s.c.) were tested. Moreover, the effects of nAChR antagonist were tested.
Results: In human study, the degree of delay discounting of gain was significantly and positively correlated with both the number of cigarettes smoked and the estimated dose of nicotine intake per day ($r=0.57$, $p < 0.05$). However, there was no relationship between smoking and the other types of discounting, probability discounting and discounting of loss. In animal studies, nicotine (0.1, 0.2, 0.4 mg/kg) significantly increased premature response, an index of impulsive behavior. Moreover, nAChR antagonist suppressed impulsive behavior.
Conclusions: 1) Nicotine increases impulsive behavior regarding gain. 2) However, nicotine does not affect impulsivity regarding loss. 3) nAChR antagonist could be an agent to suppress impulsive behavior.

Trimetazidine Revisited: Current And Future Applications Of Metabolic Modulation Of The Heart

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Background: Trimetazidine (TMZ) is an effective drug mainly used in angina pectoris. The beneficial effects of TMZ are related to the alteration of the substrate preference of the heart in favor of glucose usage and a reduction of the oxygen requirement for energy production. Increased rates of fatty acid oxidation impair contractile function in both healthy and diabetic hearts. Fatty acid oxidation dominates in Type I diabetic hearts for energy production at the expense of an increase in oxygen requirement. The objective of this study was to examine the effect of chronic treatment with TMZ on cardiac mechanical function and fatty acid oxidation in diabetic rats.
Methods: A total of 40 rats were randomly divided in 4 groups: diabetic, TMZ-treated diabetic, control, TMZ-treated control. Diabetes was induced by a single intravenous injection. TMZ (7 mg/kg/day) was supplied in drinking water for 13 weeks.
The hearts were perfused in isolated working heart mode at 11.5 mm Hg left atrial preload and 80 mm Hg aortic afterload with a modified KH solution containing 100 µU/ml insulin, 11 mM glucose, and 0.8 mM palmitate (including [9,10-^{3}H]palmitate) pre-bound to bovine serum albumin. Heart rate and peak systolic pressure were measured by a pressure transducer. Cardiac and aortic output were obtained by monitoring the flows into the left atria and from the afterload line. Palmitate oxidation was determined by measuring ^3H_2O produced from [9,10-^{3}H]palmitate using standard scintillation counting procedures. mRNA expression of long-chain 3-ketoacyl-CoA thiolase (3-KAT) was determined by RT-PCR.
Results: Coronary flow was increased with TMZ treatment. Cardiac works of diabetic hearts were significantly lower compared to non-diabetics. Fatty acid oxidation was increased in both diabetic groups. Diabetes induced the mRNA expression of 3-KAT.
Conclusions: 1) Diabetes increased the rates of fatty acid oxidation that was accompanied by deteriorated cardiac function 2-3) The inhibitory effect of TMZ on fatty acid oxidation was not detected at 0.8 mM palmitate in the perfusate. This finding along with the increase in 3-KAT expression suggest that higher enzyme expression required a higher concentration of TMZ. Also, a detailed kinetic analysis is of 3-KAT with its substrates needed which is currently performed in our laboratory.
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Comparison And Evaluation Of The Antiviral Potentials Among Different Soluble Forms Of Nectin-1 To Herpesvirus Infection

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Background: Nectin-1 is an alphaherpesvirus receptor that binds to virion glycoprotein D (gD) and mediates entry of alphaherpesviruses including HSV-1 and pseudorabies virus (PRV). We have reported that expression of soluble forms of nectin-1 provided a significant resistance against alphaherpesvirus infection to cell lines and transgenic mice. In order to compare the antiviral potentials of four different soluble forms of nectin-1 *in vivo*, transgenic mice expressing each of them were challenged with PRV.
Methods: Antiviral potentials of two soluble forms of the entire ectodomain of nectin-1 (VCChIg and VCCpIg) consisting of the entire ectodomain and the Fc portion of human or porcine IgG and two soluble forms of the first Ig-like domain of nectin-1 (VhIg and VpIg) consisting of the first Ig-like domain and the human or porcine Fc portion were compared by performing experimental infection. Survival of mice was recorded for 14 days.
Results: The average survival rates of transgenic mice expressing each of four different soluble forms of nectin-1 were as follows; 98% in VCChIg, 90% in VCCpIg 100% in VhIg and 79% in VpIg for the intraperitoneal challenge with $20LD_{50}$; 83%, 73%, 89% and 40% for 100 LD_{50}; 74%, 68%, 58% and 7% for $1000LD_{50}$; 72%, 60%, 97% and 40% for the intranasal challenge with $10LD_{50}$. In contrast, more than 90% of all control littermates in each challenge died within 14 days.
Conclusions: 1) Transgenic mice expressing each of four different soluble forms of nectin-1 showed a significant resistance to PRV infection via intraperitoneal and intranasal routes, indicating that all of soluble forms of nectin-1 are able to exert a significant antiviral effect against alphaherpesvirus infection *in vivo*. 2) Mice transgenic for a chimera that carried the human Fc portion (VCChIg and VhIg) were more resistant than those transgenic for a chimera that carried the porcine Fc portion (VCCpIg and VpIg). 3) Mice transgenic for a chimera that carried the entire ectodomain of nectin-1 were more resistant than those transgenic for a chimera that carried the first Ig-like domain, when the extracellular domains were fused to the porcine Fc portion.

Developement Of A Novel DNA Vaccine Targeting Macrophage Migration Inhibitory Factpr And Its Efficacy On Murine Models Of Inflammatory Diseases

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Background: Previous studies have demonstrated that neutralization of macrophage migration inhibitory factor (MIF) by anti-MIF antibody ameriolated the disease severity or mortality in several animal models of inflammatory diseases. This report describes a simple and effective method for active immunization aimed at eliciting neutralizing anti-MIF autoantibody, and its efficacy on the murine models of inflammatory diseases including arthritis, sepsis, and atopic dermatitis (AD).
Methods: We developed a MIF-deoxyribonucleic acid (DNA) vaccine by introducing oligonucleotides encoding helper T epitope into the cDNA sequence of murine MIF cloned in the mammarian expression plasmid pCAGGS, and administered it by in-vivo electroporation. Vacant plasmid was administered as a control vaccine (CV). Therapeutic effect of this approach against murine arthritis models induced by collagen antibody (CAIA) or in interleukin-1 receptor antagonist knockout mice (IL-1Ra KO), sepsis models induced by lipopolysaccharide (LPS) or cecal ligation and puncture (CLP), and murine AD models in DS-Nh or NC/Nga, were evaluated.
Results: Mice administered with MIF-DNA vaccine significantly raised high titers of autoantibody which reacted to native MIF. The symptoms of CAIA and IL-1Ra KO were significantly ameliorated by MIF-DNA vaccination. This approach also significantly improved the survival of mice with LPS- or CLP- induced sepsis ($p<0.05$, v.s. CV), which was associated with suppressed mRNA levels of inflammatory cytokines and toll-like receptor (TLR) 4 in the lung. Finally, MIF-DNA vaccination significantly prevented the onset of dermatitis in DS-Nh or NC/Nga ($p<0.05$, v.s. CV). Of note, this approach also significantly improved the symptoms of established dermatitis in these AD models ($p<0.05$, v.s. CV).
Conclusions: 1) MIF-DNA vaccination showed a prophylaxic effect against two models of arthritis, two models of sepsis, and two models of AD in mice. 2) MIF-DNA vaccination showed a therapeutic effect against established symptons of dermatistis in the two AD models. 3) DNA vaccine targeting MIF may provide a new strategy for the prevention and management of inflammatory diseases including arthritis, sepsis, and AD.

Transporter Mediated Drug-Drug Interactions: Modulation Of Drug Absorption By Environmental Toxicants

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Background: ABC transporter proteins are efflux pumps expressed in cell membranes, which pump their substrates out of the cytosol into the extracellular, through hydrolization of ATP. Due to their expression in barrier organs like the intestine, liver, kidney and blood-brain-barrier, ABC transporters form an important wall of defense against xenobiotics from different sources. Among others, many drugs but also environmental toxicants like pesticides have been shown to interact with ABC transporter proteins. In this study a large number of pesticides was studied for interaction with several different ABC transporters. In follow-up experiments the effect of a subset of compounds on drug absorption and cytotoxicity was studied in more detail.
Methods: A large number of pesticides was screened for interaction with human ABC transporter proteins P-gp, MRP1, MRP2 and BCRP in membrane based transporter assays (ATPase, Vesicular Transport). A subset of seven chloroacetanilide herbicides was selected and further investigated for inhibition of Pg-p in a number of methods. The effect of chloroacetanilides on the cellular efflux of calceinAM and cytotoxicity of paclitaxel, both P-gp substrates, was studied in K562-MDR cells. The transport of digoxin on Caco-2 monolayers was used as a model for drug absorption. The apical to basolateral flux of digoxin was measured in the presence and absence of chloroacetanilides.
Results: Over 50% of the tested compounds showed interaction with one or more transporters, mainly Pg-p and BCRP. Four out of seven selected chloroacetanilides showed significant stimulation of P-gp specific ATPase activity. These compounds also inhibited calceinAM efflux from K562-MDR cells and sensitized the same cells to the cytostatic agent paclitaxel. Furthermore, those compounds showing interaction with P-gp also increased the permeability of digoxin on Caco-2 monolayers up to 2.5 times.
Conclusions: This study showed interaction of structurally diverse pesticides with several human efflux transporters. The results from the experiments with chloroacetanilides demonstrate that environmental toxicants, like herbicides, can inhibit ABC transporter activity, and thereby modulate drug absorption or sensitize cells to cytostatic drugs.
Abbreviations: MDR: Multidrug Resistance, MRP: Multidrug Resistance Protein, BCRP: Breast Cancer Resistance Protein, P-gp: P-glycoprotein.

25-Years Of Sight Saving By Preventing Postoperative Scarring With Local Use Of Antimetabolites

OPHIR A

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Background: Chronic glaucoma is a progressive blinding disease. A major risk factor of disease progression is an increased intraocular pressure (IOP). When topical and laser treatments are not adequate in halting disease progression, a surgical approach is indicated. Trabeculectomy, the preferred surgical choice, is aimed to open a new, long-lasting route for outflow of intraocular fluids in order to reduce IOP. The most common cause of surgical failure is scarring of the new opening. Postoperative scarring before the local antimetabolite era occurred in 30-70% of cases, depending on the preoperative existence of factors that promote scarring. Many eyes were thus doomed to blindness.
Methods: Literature review.
Results: Since the introduction of local application of antimetabolites in 1982, initially 5-fluorouracil (5-FU) by us and later mitomycin C (MMC) by others, surgical success rate in all groups commonly exceeds 90%. As an inhibitor of fibroblasts proliferation 5-FU was injected under the conjunctiva twice daily for 14 days after surgery. Following our observations that it is also toxic to existing fibroblasts, few injections are administered and only when imminent scarring is evident. 5-FU side-effects relate to inhibition of neighboring cell proliferation, and are mild and temporary. MMC, the more potent agent, is toxic also to quiescent neighboring cells. It use could be risky, termed “time-bomb”: Gradual thinning of the postoperative conjunctival roof of the new outflow path, called the filtering bleb, led in many eyes to spontaneous ocular perforation and intraocular infection (endophthalmitis). Nowadays, following our understanding of both that MMC toxic mechanism and the modification of the trabeculectomy-MMC approach, MMC may be used safely.
Conclusions: 1. The local administration of 5-FU or MMC intraoperatively and/or postoperatively became the turning point that improved glaucoma prognosis substantially during the last decade. This led to an extensive increase in the number of glaucoma operations, that saved sight of many thousands world-wide. 2. The local use of chemotherapeutic agents has spread to avoid failure of other surgical approaches, such as ocular pterygium and lacrimal-path operations, as well as in fields other than ophthalmology, such as the use of a coated cardiac stent.

Interactions Between NE And EAAT

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Background: Excitatory Amino Acid Transporters (EAAT) are the main mechanism for its removal from the synaptic space. We have reported that alpha-1 antagonism (prazosin) prevents cocaine sensitization and it is accompanied by marked changes in Glutamate transmission (Ortiz et al 2006). We examined possible changes in EAAT acttivity brought about by cocaine exposure.
Methods: Rat cortex (CTX) and prefrontal cortex (PFC) slices were incubated with different agents for at least one hour prior to measuring EAAT activity. The slices were then incubated with 50 µM [^{3}H]Glutamate for 10 minutes. The media was the removed and the slices washed twice. The slices were then solubilized in 50 µL of 0.5 M NaOH overnight. The radioactivity was quantified after acidification with 50 µL of glacial acetic acid and addition of 1 mL Ecolume using a Beckman LS 1800 scintillation counter.
Results: Fifty micromolar NE increases EAAT activity in PFC but not in cortical slices. Cocaine increases EAAT activity in PFC slices but not cortical slices.
Prazozin also increases EAAT activity, however, propranolol (beta blocker) had no effect, nor did it prevent the effects of NE.
Conclusions: 1) Our results show modulation of EAAT activity by NE and 2) suggest non-alfa, non-beta mechanism(s).

Micro RNA As A Novel Drug For Cancer Metastasis: Suppression Of Lung Metastasis Of Osteosarcoma In *In Vivo* Model

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Background: MicroRNA (miRNA) is a small non-coding RNA consisting of 21~25 nucleotides. Recent studies disclosed that many miRNAs show some correlations with cancer progression including metastasis.
Methods: To detect candidates of miRNA in human osteosarcoma that should be implicated in lung metastasis, we performed miRNA microarray analyses on a 143B cell, a human osteosarcoma cell line showing high frequent spontaneous metastasis to lung in an *in vivo* mouse model, and comparing it with that of an HOS cell, which was a parent cell of the 143B cell and showed no spontaneous lung metastasis in mice. The detected miRNA candidates were examined to identify miRNA(s) correlated to cellular invasion by *in vitro* assay. An identified miRNA was inoculated into mice that showed spontaneous lung metastasis from primary osteosarcoma lesion at right knee joint. Expression of the identified miRNA was also examined in human osteosarcoma tissue samples.
Results: We identified nine miRNAs that were significantly down-regulated in 143B compared to HOS (P<0.01). In a matrigel invasion assay, transfection of one of those miRNAs (termed occasionally as miR-X) into 143B decreased their invasiveness. On the other hand, the miR-X poorly affected proliferation of 143B cells *in vitro*. Intravenous injection of the miR-X, but not negative control miRNA, into the spontaneous metastasis model mice successfully suppressed lung metastasis of osteosarcoma cells. Moreover, expression levels of miR-X in human primary osteosarcoma with lung metastasis after primary tumor resection showed low compared to those without lung metastasis.
Conclusion: Our data suggest that the down-regulation of miR-X correlates lung metastasis of human osteosarcoma cells by promoting cellular invasion, and that the miR-X might be a novel drug to inhibit lung metastasis of osteosarcoma cells.

Applied Reverse Vaccinology: A Meningococcal Serogroup B Vaccine For Infants

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Background and Aims: Although strains of serogroup B *N.meningitidis* are the predominant disease causing strains in many regions of the world today, there is no truly global vaccine available to prevent this particular meningococcal infection.
In a technique known as “reverse vaccinology,” potential vaccine candidates were predicted by analyzing the entire genome sequence of an invasive MenB strain. Through genetic engineering and from the investigation of 350 potential antigens, surface proteins were identified that best induced an antibody response.
Building on this genomics approach, it was possible to develop a vaccine that offered protection against multiple strains of MenB. Phase II trials have demonstrated satisfactory safety, tolerability and immunogenicity. In addition, rMenB is the first recombinant MenB protein vaccine to induce an immune response in infants.
Methods: Safety and immunogenicity of the rMenB vaccine was assessed in a 2, 4 and 6 month schedule. The immunogenicity was measured by serum bactericidal assay using human complement (hSBA).
Results: The trial demonstrated satisfactory safety, tolerability and immunogenicity. Local and systemic reactions of the vaccine candidate were similar in frequency and intensity to routine infant immunisations with the exception of fever. A moderate, short-lasting temperature rise not exceeding 39.0°C following the first dose was reported more frequently in the rMenB arm than in the control.
Preliminary analysis shows 89% (44/76-SL, ST32), 96% (5/99, ST8) and 85% (NZ98/254, ST41/44) hSBA ≥1:4 post 3rd dose against three serogroup B strains representing the major vaccine antigens.
The majority of disease causing strains worldwide express at least one of rMenB antigens.
Conclusions: rMenB vaccine is well tolerated and immunogenic against a panel of serogroup B strains in young infants when administered in a three dose schedule two months apart. This vaccine is entering phase 3 clinical trials.

Reduction Of Flubiprofen-Induced Gastric Toxicity By Prodrug Formation And Enhancement Of Oral Bioavailability Of Flubiprofen By Chitosan Complexation

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Background: Acidic nonsteroidal anti-inflammatory drugs (NSAID), including flubiprofen (FP), are poorly soluble in water and cause gastric irritation. Aims: 1) To develop a NSAID with reduced gastric toxicity by combining it with a clinically potent histamine H_2 antagonist; and, 2) To improve the dissolution characteristics for rapidly absorbed solid drug formulation using low molecular (LM) chitosan.
Methods: A conjugate of FP with N-[3-{3-(1-piperidenylmethyl)phenoxy} propyl] 2-(2-hydroxyethyl-thio) acetamide (PPA) histamine antagonist was synthesized. A complex of FP and LM chitosan (MW: approximately 750) was prepared using a kneading method. The dissolution of FP from the samples was measured using a padded method. *In vivo* absorption studies were carried out using male wistar rats, and FP was analyzed using HPLC. Gastric mucosal irritation was evaluated from the total sum of the products of length and width of each affected mucosal part from each rat.
Results: The prodrug FP-PPA was partly hydrolyzed *in vitro* in buffer (pH 1.2-7.4) in the presence or absence of pepsin and trypsin, then it was slowly hydrolyzed in gastric mucosal homogenate and quickly hydrolyzed in 10% rat plasma. The prodrug inhibited carrageenan-induced paw edema to the same extent as FP alone. The plasma concentrations of FP after oral administration of the prodrug were similar to FP alone. The prodrug formation significantly reduced gastrotoxicity in comparison with an equivalent dose of FP, whereas the coadministration of FP and PPA did not affect the gastrotoxicity of FP. The gastrotoxicity of the FP methyl ester was dependent on the drug concentration in the gastric mucosa. The dispersion of FP in LM chitosan causes a decrease of crystallinity and microcrystal size and a change of crystal lattice and microcrystal shape. The dissolution rates of FP from the LM chitosan complex were enhanced with increasing amounts of LM chitosan. The oral bioavailability of FP was improved through complex formation with LM chitosan.
Conclusions: 1) The prodrug of FP with histamine H_2 antagonist, PPA, causes less gastric damage than ester prodrugs like the methyl ester of the free drug FP. 2) The complex of FP with LM chitosan induced a faster absorption rate of FP, owing to the rapid dissolution into the GI fluid.

Complex Carbohydrate-Based Cancer Vaccines: Magic Bullets In The Making?

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Background: A hallmark of cancer establishment is heterogeneity within a single patient and even within a single organ. Heterogeneity of cancer manifests itself in many ways. In addition to all disrupted signaling pathways, the complex carbohydrates that are displayed at the cell membrane are also severely disrupted. Some are overexpressed in certain cancers more than others, while others are not properly processed. Challenging the immune system with these aberrant carbohydrate motifs seems to be a promising strategy. Vaccination against cancer though deprived of glory to date, has provided more than was originally expected of it.
Methods: Over the past decade, several major developments in chemical synthesis and manipulation of complex carbohydrates have made these complex structures available in pure single forms. This has re-opened the door to the systematic study and improvement of carbohydrate based cancer vaccines. So far, vaccination against cancer was tested in late stages of disease when everything else failed, and when patient's immune system is equally compromised. In the many vaccination trials as a means of "preventive measure against cancer relapse" complex carbohydrates have proven efficacious in prolonging patients' life compared to untreated patients.
Results: Challenging the immune system with a unimolecular multiepitopic synthesized carbohydrate-based cancer vaccine produced an antibody count that is superior to the sum of its epitopes used together as a mixture.
Conclusions: In the quest to harness the immune system to recognise and kill cancer cells using a mulitivalency strategy, made possible through major advances in organic synthesis, complex carbohydrates-based cancer vaccine strategies might be the sought after magic bullet in cancer treatement and prevention. Our synthetic efforts as well as an overview of some of our recent results will be presented.

The Ocular Penetration Of Antibiotics Using A Rabbit Model That Emulates Human Topical Dosing.

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Background: Various antibiotics are being used topically to treat ocular infections. We have developed a new rabbit model that emulates human topical ocular dosing. The model gives a level of precision that is superior to both human and animal pharmacokinetic studies, and the results correlate well with human aqueous humor levels obtained during cataract surgery. The model has been used to explore the ocular penetration and distribution of a number of commercial ophthalmic antibiotics.
Methods: Anesthetized rabbits were given a single topical dose (50 µL) of the various commercial antibiotics followed by a 30 minute controlled period that emulates the human eye with manual blinking (4 blinks/min) and a supplementary tear flow (2 µL/min). Tear samples (1 µL) were collected at 2 minute intervals throughout the dosing. The rabbits were euthanized after 60 minutes and ocular tissue samples were collected. In another study, a more frequent dosing regimen was explored. All tissue samples were extracted by sonication in water, and antibiotic concentrations were quantified using HPLC.
Results: The tear concentrations of the antibiotics decreased at a first-order rate and generally were below detectable levels after 20 minutes. The tissue levels for some of the antibiotics evaluated, 60 minutes after dosing, are shown in the table:

Antibiotic	Moxifloxacin		Levofloxacin	Gatifloxacin	Tobramycin
Dosing	Single	4 times	Single		
Tissue	Concentration (µg/g or µg/mL) Mean ± se, n = 4				
Aqueous humor	**2.2 ± 0.4**	**7 ± 1**	**0.60 ± 0.07**	**0.7 ± 0.1**	**0.03 ± 0.01**
Conjunctiva	**2.6 ± 0.1**	**16 ± 3**	**0.3 ± 0.1**	**0.4 ± 0.1**	**1.2 ± 0.2**
Cornea	**7 ± 3**	**32 ± 1**	**3.7 ± 0.6**	**7 ± 1**	**0.31 ± 0.03**
Iris-ciliary body	**1.4 ± 0.3**	**6 ± 1**	**0.37 ± 0.02**	**0.5 ± 0.1**	**< 0.1**
Sclera	**0.7 ± 0.2**	**3 ± 2**	**0.8 ± 0.7**	**1.0 ± 0.3**	**< 0.4**

Conclusions: Fluoroquinolones provide higher levels of ocular antibiotic penetration, with moxifloxacin providing the highest levels in the aqueous humor ($p<0.01$). Dosing moxifloxacin every 15 minutes for one hour prior to collection results in aqueous humor concentrations more than 3x the MIC of fluoroquinolone resistant staphylococci (2.0 µg/mL) and more than 100x the MIC of fluoroquinolone sensitive staphylococci (0.05 µg/mL).

Racial Disparity In Stroke Risk Factors: The Berlin–Ibadan Experience; A Retrospective Study

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Background: Different workers have reported racial disparities in the distribution of risk factors for stroke and stroke subtype (ischemic vs hemorrhagic). No transcultural transnational studies have been conducted to confirm and relate these disparities to one another. Our objective was to identify differences in the distribution of risk factors for stroke and stroke subtypes among urban-dwelling stroke patients in Nigeria, a developing country, and Germany, an industrialized country.
Methods: Consecutive stroke patients in Ibadan (100) and Berlin (103) were studied. Their hospital records were screened to identify documented vascular risk factors and stroke subtype.
Results: The stroke patients in Ibadan were younger than those in Berlin ($t = 4.940$, $P = 0.000$). Hypertension was significantly more common in Ibadan while cigarette smoking, dyslipidemia, atherosclerosis, and cardiac factors were significantly more frequent in Berlin. Cerebral infarction was more common in Berlin (80%) than in Ibadan (63%).
Conclusion: The risk factors associated with cerebral infarction were more frequent in Berlin. We suspect that racial disparity in risk factors for stroke may account for the difference in proportions of stroke subtype in black and white populations. Larger prospective community-based multinational multiracial studies are required to confirm these disparities and identify possible underlying genetic, dietary, and socio-economic factors.

Cytokine Profiles Of Patients With Cutaneous Leishmaniasis

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Background: The *Leishmania* spp. are obligate intracellular protozoa that cause a spectrum of diseases, including cutaneous, mucocutaneous, and visceral leishmaniasis, in tropical or subtropical countries. Sanliurfa is an area highly endemic for cutaneous leishmaniasis caused by the protozoan *Leishmania tropica* and it has been for many years an important focus in Southern Anatolia of Turkey. This research was planned to detect the cytokines in sera of patients with active cutaneous leishmaniasis in Sanliurfa.
Methods: In this study, the cytokine measurements were made in sera of 25 patients with active cutaneous leishmaniasis, before and after the sodium stibogluconate therapy and 25 healty control people. Cytokines such as IFN- γ, TNF-α, IL-2, IL-4, IL-6, IL-10 and IL-12 were measured in groups and the results were compared.
Results: IL-2, IL-4, IL-6, IL-10, IFN- γ levels were found higher in active cutaneous leishmaniasis than control group, while no differences in IL-12 levels were found. IL-4, IL-6, IL-10, IFN- γ levels were found lower at post treatment compared to pretreated patients with cutaneous leishmaniasis.
Conclusion: Following up the cytokine levels of these patients with cutaneous leishmaniasis can give an idea on the course of the disease and can contribute to schematize cytokine treatment

A Molecular Perspective On Integron-Associated And Transferable Antibiotic Resistance In Clinical And Aquatic Isolates Of Gram-Negative Bacteria In Northern Region Of Turkey

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Background: We analyzed the data obtained from our earlier studies concerning the mobile genetic elements such as conjugative resistance plasmids and class 1 and class 2 integrons in multi-drug resistant Gram-negative pathogens isolated from different aquatic or clinical environments in the northern region of Turkey.
Methods: Disk diffusion and agar dilution methods were used in antibiotic susceptibility testing. Transferable antibiotic resistance was detected by conjugation assays. Class 1 and class 2 integron gene cassettes were screened and then described by sequencing. Sequences were compared to the current GenBank databases using the BLAST suite of programs. CLUSTALW amino acid sequence alignments were produced for comparison.
Results: Resistance to ampicillin, tetracycline, streptomycin, trimethoprim and sulphamethoxazole was commonly high in multi-drug resistant organisms from both aquatic and clinical isolates, and much of them were transferable traits carrying on conjugative plasmids. The gene cassettes such as the families of *aadA* and *dfrA* inserted in integrons were commonly shared between aquatic and clinical isolates, and some of them were novel gene cassette arrays and new alleles.
Conclusions: We conclude that further molecular epidemiological investigations should be committed to monitor for these antibiotic resistance genes in various environments in the other regions of Turkey to take precautions for slowing down the progression of antibiotic resistance evolution country-wide.

Circulating epithelial tumor cells (CETC) allow gene analysis of the residual tumor burden individual chemosensitivity testing and monitoring of the adjuvant setting in a curative

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Background: Although most malignant tumors have no detectable metastases at diagnosis, most patients do not die from the primary tumor but from metastases during the subsequent course of disease. Circulating epithelial tumor cells (CETC) emanating from the primary tumor are assumed to be the source of such metasetases. Understanding the composition and the genetic variability of CETCs, chemosensitivity of remnant CETCs before and after specific therapies and monitoring during systemic therapy will further our understanding of therapy reponse and resistance.
Methods: Monitoring of neoadjuvant systemic therapies included 50 lung cancer patients and 75 breast cancer patients, of adjuvant therapies 200 breast cancer patients, and chemosensitivity testing has been performed in over 100 patients. CETCs were detected and monitored from peripheral blood of cancer patients requiring not more than 1ml of blood by staining with fluorochrome labeled antibodies to EpCAM and CD45 antigen by laser scanning cytometry or automated image analysis. Reanalysis for genetic aberrantions was performed by fluorescence in situ hybridization and single cell PCR and in vitro cytotoxicity testing by analysis of specific tumor cell killing by chemotherapeutic agents.
Results: Viable epithelial cells, suspect of tumor origin, were detectable in more than 90% of tumor patients with a good correlation between tumor size and the number of CETCs before therapy indicating that such cells are released during tumor growth and can be disseminated during diagnostic and therapeutic interventions. CETCs from individual patients showed a wide genetic heterogeneity of e.g. the her2/neu amplification and this may in the future be used for optimizing therapy. Longitudinal monitoring allowed supervision of the action of therapeutic agents on CETCs in vivo. Neoadjuvant therapy (with the primary tumor still present) first eliminated CETCs by but with continuing tumor disintegration cells were again released into circulation. Numbers could vary up to 10 thousand fold. Monitoring of the adjuvant therapy success revealed that an increase in CETC numbers more than tenfold towards the end of therapy was a strong predictor of early metastasis formation. The correlation between in vitro sensitivity of CETCs and therapy response is now under investigation.
Conclusions: Analysis of CETCs responsible for fatal metastasis formation, of their gene endowment, their sensitivity to chemotherapeutic agents and their in vivo response to therapy allows, insight into the mechanisms of tumor cell killing and resistance and will greatly contribute to individualized therapeutic approaches

A biomarker panel for acute graft versus host disease

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Background: No validated biomarkers exist for acute graft versus host disease (GVHD), the major complication of allogeneic hematopoietic cell transplantation.
Methods: We screened plasma with antibody microarrays for 120 proteins in a discovery set of 42 transplant patients. We then measured the levels of the best biomarkers from the discovery set by sequential ELISA to create a composite biomarker panel that we tested in samples from 424 transplant patients randomly divided into training (n = 282) and validation (n = 142) sets.
Results: Analysis of 23 proteins in the discovery set revealed eight potential biomarkers. Logistic regression analysis of these eight proteins in the samples of the training set determined a composite biomarker panel of four proteins (interleukin-2-receptor–alpha, tumor-necrosis-factor-receptor-1, interleukin-8, and hepatocyte growth factor) that optimally discriminated patients with and without GVHD. The area under the receiver operating characteristic curve distinguishing these two groups in the training set was 0.91 [95% confidence interval, 0.87 - 0.94] and 0.86 [95% confidence interval, 0.79 - 0.92] in the validation set. A model utilizing protein levels with 95% specificity for GVHD in the training set provided 94% specificity in the validation set. In patients with GVHD, Cox regression analysis revealed that the biomarker panel independently predicted survival independently of GVHD severity ($p < 0.001$, Table 1).

Table 1: Association of Maximum GVHD Grade and Biomarker Panel with Overall Survival

	Univariate		Multivariate	
	Hazard Ratio	p value	Hazard Ratio	p value
Maximum GVHD Grade (1/2 vs 3/4)	2.35	<0.001	2.11	0.001
Biomarker Panel[#]	2.46	<0.001	2.43	<0.001

[#]For subject with levels of each biomarker in the panel that are 1.5 times the median versus a subject with levels of each biomarker at the median.

Conclusions: A panel of four biomarkers can confirm the diagnosis of GVHD in patients at onset of clinical symptoms of GVHD and provide prognostic information independent of GVHD severity.

Predicting the Impact of Hepatic Transporters on the Pharmacokinetics of Statins in the Liver

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Background: The statins form a class of hypolipidemic drugs used to lower cholesterol levels in people with or at risk of cardiovascular disease by inhibition of the HMG-CoA reductase enzyme. The enzyme is found in the smooth endoplasmic reticulum within the hepatocytes of the liver. Statins are known to be transported into hepatocytes thus raising their intracellular concentration. The ability to predict the free concentration of a drug within the hepatocytes of the liver will lead to a better understanding of the pharmacokinetics, pharmacodynamics and potential drug-drug interactions of statins.
Methods: This study included the drugs atorvastatin, cerivastatiin and indomethacin as a control. Disposition of these compounds was measured in isolated rat hepatocytes and the data fitted to a kinetic model incorporating active uptake, permeation, binding and metabolism. Parameters from the hepatocyte kinetic model were transposed to an in vivo kinetic model and used to predict the pharmacokinetics of the drugs including the all-important free concentration within the hepatocytes of the liver. These predictions were compared to measured in vivo pharmacokinetics (plasma, liver and muscle) from rat bile duct cannulated animals.
Results: Use of the hepatocyte model to estimate the ratio of intracellular to extracellular steady-state free-drug concentrations demonstrated the strong influence of active uptake on the kinetics of atorvastatin (18:1) and cerivastatin (8:1), in comparison with indomethacin (3.5:1). Indomethacin, however, was shown to have a higher uptake clearance (599 ± 101 µl/min/106 cells) than atorvastatin (375 ± 45 µl/min/106 cells) and cerivastatin (413 ± 47 µl/min/106 cells). The high passive permeability of indomethacin (237 ± 63 µl/min/106 cells) clearly negated the effect of the active transport on the overall disposition. Hepatic clearance was well predicted by the analogous in vivo model, in contrast to predictions based on standard methods. Volume of distribution was well predicted for indomethacin and predicted reasonably for atorvastatin and cerivastatin and higher than might be expected for an acid compound. Furthermore, the terminal half-life predictions for all 3 compounds were within two-fold of the observed values.
Conclusions: 1) The disposition of atorvastatin and cerivastatin in isolated rat hepatocytes can be fitted to an hepatocyte model that incorporates active uptake, permeation, binding and metabolism. 2) In vivo pharmacokinetics in plasma, liver and muscle can be predicted from disposition in isolated hepatocytes.

HPLC and MALDI TOF MS analysis of novel antileishmanial compounds from *Quassia amara*

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Background: Quassinoids are used in folk medicine for centuries and have antileukemic and antimalerial activities. Aims: 1) To charecterise and quantify quassinoids from *Quassia amara* bark tissue using HPLC and MALDI TOF 2) To assess antileishmanial activity of purified quassinoid and neoquassinoid.
Methods:
Lypholysed methanolic extract of dried, powdered 1.0g bark tissue was dissolved in 1ml methanol and filtered with 22µ filters. A HPLC DAD (LC-20 AT Liquid Chromatogram) was performed on C-18 column with a linear gradient mobile phase of methanol: water; for detection of quassinoids. HPLC elutes were collected, pooled, lypholysed and stored. MALDI TOF analysis of elutes dissolved in 100µl of 5% formic acid: acetonitrile buffer (1:2) was done on a Bruker Auto Flex II TOF/ TOF MALDI mass spectrometer in positive ion reflector mode using delayed extraction and nitrogen laser (337nm).
Cytotoxicity test was performed by MTT assay of the elutes at 1-500ng^{-ml} and 1-250$\mu g^{/ml}$. The effects of two quassinoids on viability of *Leishmania donovani* promastigotes were assessed by monitoring MTT metabolism after 96h cultured in presence of 1-100$ng^{/ml}$ compound. Intracellular parasitic load of amastigotes/ 100 macrophages was determined by Giemsa staining of *L. donovani* infected peritoneal macrophage cells incubated with the extracts at 1-100µg concentration.
Results: 8.28μg^{-mg} Quaasin & 7.66μg^{-mg} neoquassin were detected at 14min and 21-22min, respectively from *Q. amara* bark as confirmed by retention time (HPLC) and UV-vis spectral (DAD) analysis with authentic compound. On MALDI TOF analysis showed protonated quassin at *m/z* 389.249 and that of neoquassin at *m/z* 391.316.
For antilieshmanial assay, quassin and neoquassin was found to be non–cytotoxic to $25\mu g^{-ml}$. The LD_{50} value of quassin and neoquassin for *L. donovani* promastigotes was $62\mu g^{-ml}$ & $0.098\mu g^{-ml}$, respectively. The ED_{50} value of quassin and neoquassin in reducing the intracellular parasitic load was $12.63\mu g^{-ml}$ & $8.27\mu g^{-ml}$, respectively.
Conclusions: 1) Significant amount of quassin and neoquassin was detected from *Quassia amara* bark tissue on HPLC analysis. 2) This is the first report on MALDI TOF analysis of quassinoids, a soft ionisation technique that is increasingly being used for the detection of complex organic molecules. 3) Neoquassin was found to be more effective than quassin as an antileishmanial agent.

Surface-modified polyamidoamine (PAMAM) dendrimers for site-specific gene delivery

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Background: Major limitations of using the PAMAM dendrimers for *in vivo* gene delivery application are low transfection efficiency, lack of target specificity, and limited transport into the nucleus of the target cells. Herein we report preparation and evaluation of ornithine-conjugated PAMAM dendrimers and their potential for cancer cell-specific gene delivery.
Methods: Ornithine-conjugated PAMAM dendrimers were prepared by Fmoc synthesis. A comparative gene transfection study between PAMAM dendrimers and the surface modified dendrimers was conducted in HEK 293T, GM7373, NCI H157G cell lines. Cytotoxicity of the dendriplexes was tested in HEK 293T cells by MTT assay. Effect of excess of ornithine (100 µM) on transfection efficiency of the ornithineconjugated PAMAM dendrimers was investigated. A comparative transfection study in polyamine transport deficient (NCI H157R) and polyamine transport efficient (NCI H157G) cell lines was performed to confirm the role of the polyamine transporter system (PAT) in the dendriplex uptake.
Results:
1H NMR and MALDI-TOF spectral analysis showed that about 60 molecules of ornithine (PAMAM-ORN60) were coupled to a PAMAM dendrimer. Comparative study between cancer cell (NCI H157G) and HEK 293T cells showed higher transfection efficiency of PAMAM-ORN60 dendriplexes in cancer cells than normal cells. Cytotoxicity assay has shown that dendriplexes prepared at N/P of 10 were safe at concentrations below 50 µg/mL. Transfection efficiency significantly increased with increase in generation number and degree of ornithine conjugation. Transfection efficiency of the $PAMAM_{G4}$-ORN60 dendrimers decreased in presence of excess of ornithine while there was no effect on the parent $PAMAM_{G4}$ dendrimers. Transfection efficiency of $PAMAM_{G4}$-ORN60 was significantly low in NCI H157R (31.6±12.5%) as compared to NCI H157G cell line (63.1±8.6%).
Conclusions: Conjugation of ornithine significantly increased the transfection efficiency of PAMAM
dendrimers. $PAMAM_{G4}$-ORN60 dendriplex uptake was higher in cancer cells as compared to HEK 293T cells. Many tumor types have been shown to contain elevated polyamine levels and an activated PAT system. As the results demonstrated the role of PAT in the uptake of $PAMAM_{G4}$-ORN60, they may serve as potential cancer cell-specific gene carriers.

Targeted and Multifunctional Dendritic Polymers: Magic Bullets for Drug and Gene Delivery

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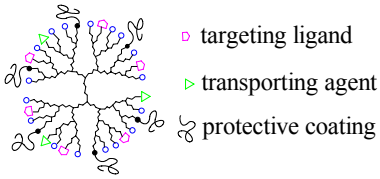
Background: A significant number of molecules fail to be commercialized due to inability to be delivered to the appropriate tissues. Such bioactive molecules lack blood solubility, stability, tissue specificity and transport ability through cell membranes. For addressing these problems drug carriers based on dendritic polymers have been developed.
Methods: Consecutive functionalization of these polymers leads to the preparation of multifunctional dendritic polymers which share the properties of drug delivery and controlled release. Thus, dendritic polymers have been developed bearing targeting ligands, which in addition to exhibiting the property of multivalency they are also complementary to cell receptors. Protective groups have also been introduced which prolong their circulation in biological fluids. Another crucial parameter for effective drug delivery systems is their transport through cell membranes. Finally, nanocavities have been tailored in such a way that drug release is triggered by changes in the biological environment.
Results: A schematic representation of a multifunctional dendrimer is shown below. Each group plays a specific role. Thus, specificity has been achieved by targeting ligands while transport through cell membranes has been achieved by molecular transporting moieties. Enhanced water solubility, decreased toxicity, biocompatibility, stability and protection has been achieved by functionalizing dendritic polymers with poly(ethylene glycol) chains. Finally, cationization of dendrimers induces the interaction with genetic material for the formation of complexes employed in gene therapy.



Conclusions: Designed functionalization of dendritic polymers scaffolds results in the preparation of non-toxic nanocarriers of significant encapsulating capacity, specificity to certain biological cells and transport ability through their membranes.

Potential Reproductive and Developmental Damage Induced by Metronidazole

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Background: Parasitic illnesses increase all over the world and metronidazole (MTZ) is the well-established antiprotozoal and antibacterial agent usually administered to children and adults at the reproductive age. Aim: To evaluate MTZ 1) as inductor of reproductive damage in *Rattus norvegicus* females and CFW male mice. 2) as teratogenic in *Drosophila melanogaster*, since the fly and vertebrates show similar developmental mechanisms.
Methods: Reproductive effects in rats were evaluated by scoring 70 adult females (450 g/bw) treated with 250, 500 and 1000mg/kg/bw/day for 7 days. They were mated after treatments and sacrificed at 21 days of gestation. To study the stages of the seminiferous epithelial cycle and spermatozoa morphology, 60 days old male mice received an MTZ dose of v.ip 130 mg/kg/bw. Pachytene spermatocytes, spermatids stages and spermatozoa were analyzed. Developmental effects were studied by allowing wild-type female flies to lay eggs for 24 hr in media with MTZ at 0, 500, 1000, and 2000 µg/ml. Emerging flies (400 to1000 for each concentration) were examined. Control series (C) kept in standard conditions were always run.
Results: In female rats MTZ did not affect pre-implantation deaths but increased the frequency of post-implantation deaths (C=3.2%, T=14.9, 16.1, 20.5%) and of dominant lethals (C=3.9, T=12.0, 13.2, 17.8%; *P<0.05; Mann-Withney U test*) In exposed mice cellular composition and number of stages in the seminiferous tubules were not altered, but the spermatozoa morphology was severely affected (C=39.7±1.0, T=122.9±3.6; *P<0.0000; ANOVA Test*). In flies, 1000 and 2000µg/ml MTZ-treated series showed higher frequencies of total abnormalities (C=1.6%, T=2.8, 3.9, 3.2%; *P<0.05; χ^2 Test*)
Conclusions: 1) In female rats treatments affected post-implantation death and induced dominant lethals, but abnormal offspring was not increased, probably because conceptions with aberrations are eliminated during post-implantation period. 2) The alteration of spermatozoa morphology by MTZ could represent a potentially serious threat to the normal fertilization process. 3) The morphogenetic alterations induced in Drosophila could indicate a potential developmental toxicity of the drug. 4) side effects of MTZ have to be considered since they represent a conceivable thread regarding fertility or development.

Structure Based Design of Second Generation PDE5 Inhibitors

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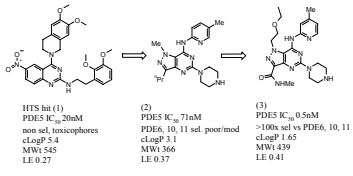
Background: The clinical knowledge gained from the pioneering PDE5 inhibitor sildenafil and subsequent agents, has highlighted the potential of PDE5 inhibition for treatment of additional indications beyond male erectile dysfunction. Such indications would be best treated by highly selective agents suitable for chronic once daily oral dosing. This talk details the discovery and progression of a pyrazolopyrimidine PDE5 inhibitor series designed to display inherently good physicochemistry, and targeting a chronic once daily treatment goal.
Methods: Despite a wealth of literature in the PDE5 field, at the outset of the programme we felt that no single chemotype had the profile we were looking for. Thus, at the point of initiation of our chemistry program, we proposed a set of criteria that would enable us to be confident a chemical lead would be likely to result in a clinical candidate with the desired profile. These criteria included measures of potency/selectivity (PDE5 <50nM & >10-fold selective over all other PDEs), physicochemistry (MW<400, LogD 1-2) and adsorption/metabolism (well fluxed, predicted human half-life >12 hours), which were used to track progress at each stage of the project. In targeting these objectives, we set out to harness three technologies (PDE5 structural information, high throughout screening (HTS) and parallel synthesis) to speed our progress. Ligand efficency (LE, log of potency divided by heavy atom count) together with cLogP were used as key measures of compound assessment rather than potency alone.
Results: Under the guidance of co-crystal structural information, a non-selective HTS hit (**1**) with poor physicochemistry was initially modified using parallel chemistry to give a lead compound (**2**) that established a new pyrazolopyrimidine PDE5 inhibitor series. Notably, (**2**) displayed physicochemistry compatible with a long plasma half-life, and wide chemical scope. Subsequent optimisation of (**2**) using crystal structure information to guide design, led rapidly to a highly potent and selective PDE5 inhibitor (**3**). Optimisation of (**3**) will be described, and continued focus on physical properties led to a selective series with good pharmacokinetics.



Conclusions: In summary, by applying a combination of structure based drug design and rigid physicochemical criteria, we were able to identify a new PDE5 inhibitor series with wide chemical scope. Utilisation of co-crystal structural information and the wide scope enabled rapid transformation into potent and selective leads. Focus on retention of inherently good physicochemistry throughout has enabled the identification of a range of potent and selective PDE5 inhibitors with good physical properties. These leads led to a physicochemically optimised amide with the potential for once daily pharmacokinetics in man.

Targeting the vulnerability of cancer cell mitochondria to selectively induce apoptosis: **Evaluating the efficacy of Pancratistatin as a non-toxic anti-cancer agent**

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Despite the aggressive research efforts to find selective anti-cancer chemotherapeutics, cancer remains unconquered. The major difficulty with the treatment of cancer is the non-specificity with which chemotherapy kills cells. Many current treatments, including radiotherapy, are damaging to both cancerous and non-cancerous cells. Non-specific damage causes harsh side effects and DNA mutations that increase the possibility of cells becoming cancerous. Pancratistatin is a natural compound that was isolated from the spider lily in 1992, by Petit *et al*., and has been shown to have anti-cancer properties. We have recently demonstrated that while Pancratistatin induces apoptosis (programmed cell suicide) in cancer cells it does not affect non-cancerous cells. We have also demonstrated the non-genotoxic nature of Pancratistatin; its ability to kill cancerous cells without targeting their DNA. We have investigated the specificity and biochemical mechanism of action of Pancratistatin; our results indicate that Pancratistatin specifically and effectively induces apoptosis in human prostate cancer, breast cancer, neuroblastoma, melanoma and leukemia cell lines. Interestingly, we have demonstrated that Pancratistatin targets the mitochondria of cancer cells. Mitochondria from non-cancerous cells are not affected by this treatment, indicating that vulnerability to this compound is limited to cancer cell mitochondria. Our preliminary *in vivo* results with human colon and prostate cancer xenotransplants in immuno-compromised mice have indicated that Pancratistatin inhibits tumor growth and is well-tolerated at the effective dose. These results reveal a new opportunity for the development of chemotherapy that targets mitochondria of cancer cells and advance our knowledge of a novel mechanism of action for Pancratistatin.

The role of corticosteroids, male and female sex hormones in a complex with apolipoprotein A-I in the regulation of gene expression

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Apolipoprotein A-I (apoA-I) is a universal transport species for corticosteroids, female and male sex hormones. This protein performs the addressable delivery of hormones into cell nuclei. The initial unit of this mechanism is represented by receptor mediated endocytosis. In cell nucleus, a hormone – apoA-I complex interacts with gene promoters comprising $(GCC)_n$ sequences and initiates their expression. However, the biological activity is inherent only in the steroid hormones that have a reduced Δ^4,3-ketogroup in the A-ring. The molecular-biological methods, IR spectroscopy and small-angle X-ray scattering were used to reveal the mechanism of gene expression in a cell culture and eukaryotic DNA. The mechanism is related with competitive rupture of hydrogen bonds in complementary GC-pairs with participation of the OH-group in the third position of hormone A-ring. Further rupture of hydrogen bonds is caused by hydrophobic interaction between nitrous base rings and hydrophobic regions of apoA-I. This creates conditions for deposition of RNA-polymerase onto DNA matrix and enhancement of gene expression. In a complex with apoA-I, such effect is observed with tetrahydroderivatives of steroid hormones, dehydroepiandrosterone, its sulfated form (dehydroepiandrosterone sulfate), pregnenolone, androsterone, and other hormones.
In the organism, the A-ring Δ^4,3-ketogroup of steroid hormones is reduced in resident macrophages that have a high 5α- and 5β-reductase activity. The macrophages entrap steroid hormones in HDL. The entrapment occurs via receptor mediated endocytosis. In secondary lysosomes of the cells, lipoproteins are disintegrated, which is accompanied by apoA-I release and reduction of Δ^4,3-ketogroup of steroid hormones. Both compounds form the biologically active complex, which is secreted to interstitial space due to exocytosis. Further the complex is transferred to the somatic cell nuclei, where it participates in the enhancement of gene expression. The mechanism revealed in our work is nonspecific. However, it becomes specific when occurring in the cells of target organs of the corresponding hormones, for example, in lymphocytes, reproductive organs, hormone-dependent neoplasms, etc.

The trypanicide Effects of Amiodarone and the Azoles: From the Skin to the Heartbeat of a Continent.

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Background: Cutaneous leishmaniasis and Chagas' disease are among the most prevalent endemic zoonosis in the Latin-American continent. The occurrence of mixed infections of *Trypanosoma cruzi* and *Leishmania* species is becoming a common feature in Central and South America due to overlapping endemic areas. Unfortunately, the possibilities for treating flagellated kinetoplastid infections are still very limited and most of the available drugs exhibit severe side effects. Although the development of new drugs against Leishmaniasis has markedly improved in the last years, the tendency is still to employ antimonial compounds. On the other hand, Chagas' disease treatment is only available for the acute phase with still no effective therapeutic options for chronic stage disease.
Nowadays several classes of compounds targeting validated biochemical pathways of the parasites are available, some proved to be useful in animal models, others widely used in other human pathological conditions and others still under clinical trials.
The following work extensively documents the diagnosis, successful treatment (using non-conventional approved drugs), and follow up of two complicated clinical cases of these diseases. The objective of this work is to present to the scientific community these two magic bullets as an alternative therapeutic option to treat these neglected diseases.
Methods: Two case reports are described. The first patient is a case of concurrent borderline disseminated cutaneous leishmaniasis and Chagas' disease, and the second a case of chronic Chagas' cardiomyopathy. Patients were diagnosed and followed up using conventional diagnostic methods as well as novel highly specific and sensitive recombinant proteins (PGR31-HIS, PGR30-HIS, anti-rTc24) for *T. cruzi*. Both patients were initiated on amiodarone and itraconazole, which, despite their classic therapeutic use, also target specific metabolic aspects of the parasite.
Results and conclusions: Complete cure was achieved by using these drugs**.** In both cases patients were demonstrated to be clinically and serologically cured. As far all authors are concerned these are the first case reports which reveal amiodarone's anti *T. cruzi* and anti-*Leishmania* effect in human subjects.

Receptor Protein Tyrosine Phosphatase Beta/Zeta as a Possible New Target to Regulate Endothelial and Tumor Cell Migration

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Background: Receptor protein tyrosine phosphatase β/ζ is a member of the receptor protein tyrosine phosphatases, characterized by the presence of an N-terminal carbonic anydrase-like domain, a fibronectin type III domain and a serine, glycine-rich domain for chondroitin sulfate attachment in the extracellular region. It exists as four isoforms: long and short receptor forms and long and short secreted proteins containing the extracellular domains of the long and short receptor forms respectively. The cytoplasmic domain of RPTPβ/ζ contains two tandemly repeated phosphatase domains, of which only the membrane proximal is catalytically active, as well as a C-terminal PDZ-binding motif, through which it interacts with other PDZ domain-containing proteins. It is expressed in the nervous system and is involved in neuronal cell migration, differentiation, circuit formation and regulation of neuronal plasticity. RPTPβ/ζ is known to bind several extracellular matrix proteins and cell adhesion molecules and it is a receptor of the heparin-binding growth factors pleiotrophin and midkine. We have studied expression of RPTPβ/ζ in primary endothelial cells and several tumor cell lines, as well as its involvement in endothelial and tumor cell migration.
Methods: A combination of methods was used, including immunoprecipitation and western blot analysis, migration assays, siRNA, immunofluorescence and confocal microscopy.
Results: RPTPβ/ζ is expressed in endothelial, as well as diverse tumor cells and in all cases interacts with pleiotrophin and mediates its effect on cell migration. Interestingly, the effect of RPTPβ/ζ on cell migration can be modulated by its interaction with other cell surface molecules, such as integrins. Moreover, RPTPβ/ζ seems to mediate cell migration induced by diverse factors, such as hydrogen peroxide, nitric oxide, aprotinin and vascular endothelial growth factor, an effect that may or may not be dependent on pleiotrophin.
Conclusions: RPTPβ/ζ seems to be an interesting target in order to limit endothelial and tumor cell migration.

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Neurosteroids in the treatment of neurodegeneration

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Background: The brain has the ability to convert cholesterol to steroids, otherwise known as 'neurosteroids'. Various studies using either pharmacological tools modulating endogenous neurosteroid levels and behavior or correlation of neurosteroid levels with neuropathology suggested that neurosteroids could be targeted to treat disturbances in the nervous system. Alzheimer's disease (AD) is a yet incurable degenerative neurological illness characterized by memory loss. We reported that 22*R*-hydroxycholesterol (22ROHC), a steroid intermediate formed during the synthesis of pregnenolone from cholesterol, is present at lower levels in the hippocampus and frontal cortex of AD patients than in corresponding tissue from age-matched controls. 22ROHC was then found to protect against β-amyloid ($A\beta_{42}$)-induced neurotoxicity. $A\beta_{42}$ as well as the formation of Aβ oligomers and amyloid deposits has been linked to AD pathology.
Methods and Results: Because 22ROHC is a rapidly metabolized intermediate in pregnenolone biosynthesis, the stable naturally occurring heterospirostenol, (22*R*,25*R*)-20α-spirost-5-en-3β-yl hexanoate (caprospinol), was identified by in sislioc screening of commercial libraries as the lead substitute. Caprospinol was found to protect against $A\beta_{42}$-induced neurotoxicity in vitro. This steroid binds to $A\beta_{42}$, inhibits the formation of neurotoxic amyloid oligomers, prevents $A\beta_{42}$ from reaching neuronal mitochondria, and protects mitochondrial function against direct insults. To investigate the in vivo efficacy of this compound we used a rat model of AD, which recapitulates the histopathological and cognitive phenotype of AD. Caprospinol treatment of diseased rats restored spatial memory, as assessed using Morris water maze tests. This recovery of cognitive function was accompanied by a reduction in hippocampal amyloid deposits and neurodegeneration. In parallel studies caprospinol administration resulted in an important accumulation of the compound at the forebrain demonstrating its ability to cross the blood-brain barrier. Caprospinol does not bind to any known steroid hormone receptors and is devoid of acute and 3-month toxicity in rodents.
Conclusions: These results position caprospinol as a promising drug candidate for AD treatment.

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Capecitabine-associated coronary vasospasm: a case report

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Capecitabine (Xeloda) is an oral 5-Fluorouracil pro-drug used in the treatment of two of the commonest cancers: breast and colorectal. Here, we report a 43 year old woman with metastatic cancer of the sigmoid colon who developed cardiac chest pain 5 days after starting capecitabine therapy. Capecitabine-induced cardiac symptoms have previously been reported, but infrequently. In the main they have documented pain and ECG changes associated with exercise. This case report is of a patient with minimal cardiac risk factors, who suffered ischaemic cardiac pain with widespread ECG changes at rest which resolved with a nitrate infusion. We propose coronary vasospasm as the probable mechanism for the cardiac ischaemia and dramatic ECG changes. Capecitabine is now in widespread use and so physicians will encounter an increasing number of patients using this therapy. In light of this, we think it important that doctors in Emergency and Acute Medicine are aware of its treatable cardiac side-effects.

Isolation, purification, partial characterization, biochemical properties and stability of two novel antimicrobial peptides produced by *Pediococcus* strains

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Background: Fermentation broths (in MRS medium) of *Pediococcus acidilactici* NRRL B5627 and *P. pentosaceus* SM-1, were found to exhibit strong antimicrobial activity against a wide range of food spoilage and food born pathogen bacteria, including *Clostridium* and *Listeria* spp. The antimicrobial factor in each case was identified to be a peptide.
Methods: The broths were subjected to repeated tricine-SDS-polyacrylamide gel electrophoresis until the antimicrobial factor was identified by overlaying the gel with nutrient agar in which the indicator microorganism *Micrococcus luteus* was embedded. Following gel excising and destaining, the proteins of interest were eluted directly into an aqueous solution of formic acid/water/2-propanol (1:3:2/v). Molecular mass determination by electrospray ionization mass spectrometric analysis (ESI-MS) revealed a 3.660 Da peptide in the case of *P. acidilactici*, while a 5.370 Da peptide in the case of *P. pentosaceus*.
Results: N-terminal sequencing determined by Edman degradation in both cases showed that the peptides have 19 amino acid residues and the consensus sequence of -YGNGV- near the N-terminal. The last characteristic classifies the peptides to the class IIa of bacteriocins, known as pediocins. The names of pediocin SA-1 from *P. acidilactici* and pediocin SM-1 from *P. pentosaceus* were assigned to the isolated peptides. The purified pediocins were examined for their sensitivity to proteolytic enzymes and for their stability to cold, heat and pH treatments (the parameters ranging widely). The most remarkable characteristics were the stability to heat treatment -both peptides were heat stable for up to 60 min at 121^{o}C and to cold storage- peptides remained stable for one year at -80 ^{o}C and -20 ^{o}C. Pediocin SA-1 remained active after treatment with trypsin, α-chymotrypsin, pepsin and papain, but lost its activity with proteinase K, while pediocin SM-1 lost its activity in most cases. The mode of action of both pediocins was found to be bactericidal. Fermentation kinetics studies in bioreactors revealed the primary metabolite nature of the peptides.
Conclusions: Antimicrobial peptides produced by lactic acid bacteria are believed to be a potential answer to the growing problem of resistance to conventional antibiotics. The fact that the two isolated pediocins are produced by well-known food grade bacteria, makes them important candidates for use as biopreservatives. The fact however, that the producer bacteria belong to the human intestinal lactic acid bacteria, makes them attractive targets for further research with the aim of the production of novel antimicrobial drugs or innovative drug delivery systems.

A Novel, Safe, and Effective Clinical Treatment to Eliminate Resistance in Ectoparasites

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Background: Head lice have been on the increase for over a decade, in large part due to the fact that they have become resistant to conventional treatment. Head lice *(Pediculus capitis)* feed every 4 to 6 hours on human blood by attaching to the scalp .The adult female louse usually lays eggs (nits) near the scalp and cements them to a hair shaft. If they are not killed or
removed, nits hatch in 7-10 days and propagate the infestation. We are studying a botanical derived product (BGC20-582 Lice Treatment Gel) that kills both lice and their eggs.
Methods: Pediculicidal activity (*in vitro*) was evaluated for BGC20-0582 in concentrations of 0% (vehicle), 2.5%, 7.5%, 10.0% and 12.5%. NIX®, an FDA approved 1% permethrin product, was used as an active control and water as a negative control. Five centimeters disks were cut from 100% cotton towels and placed in the bottom of 15 X 60 mm Petri dishes. Each formulation was measured at 0.7cc and spread across the bottom of the disk. Adults lice and nymphs, were distributed evenly between test samples and controls. Pediculicidal activity was observed over an exposure period of 5 minutes to 1 hour for each treatment. Ovicidal activity (*in vitro)* was evaluated with the same concentrations and controls. Groups of 10 hairs, each with a viable egg, were cut to 2-cm long strands and grouped together at one end with small adhesive labels. Each group was immersed in a test product for 30 minutes, and then rinsed with purified water and air dried prior to being transferred into 1 dram sterile vials and labeled accordingly. The eggs were placed in an incubator for two weeks with 80 degree F. temperature and relative humidity of 70%-80%. Ovicidal activity was determined by observing the number of eggs failing to hatch following the 14 day incubation period.
Results: Based on preclinical study results it is proposed that the required concentration and exposure time for BGC20-0582 to achieve maximal pediculicidal and ovicidal activity in vitro is treatment with a concentration of 10% for a period of 30 minutes.
Conclusion: We have just concluded a 4 arm, randomized double blinded-placebo controlled dose ranging study on approximately 230 subjects between 6 months of age and 70 years. This population is known to have permethrin and pyrethrin resistant head lice. The results are being analyzed and will be available shortly.

Erythropoiesis-stimulating agents may improve survival in low-risk MDS patients

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Background: Correction of anemia is generally the primary therapeutic goal in low risk MDS. EPO and its derivative darbepoietin alfa (DAR) are important treatments of anemia in low- risk MDS. The objectives of the present study were (i) to confirm prognostic factors of response and duration of response to rEPO+/- G-CSF in 403 MDS patients from the GFM (ii) to compare outcome of our cohort (in terms of progression to AML and survival) to that of untreated patients included in the International MDS risk Analysis Workshop(IMRAW) database (P.Greenberg) that was used to establish IPSS.
Methods: 403 patients with MDS according to FAB criteria from 25 French and Belgian hematological centers of the GFM who had received rEPO (ie epoetin alfa or beta, or Darbepoietin DAR) +/-G-CSF treatment at weekly doses of 60000 U for epoetin and 300µg for DAR during at least 12 weeks between 1998 and May 2006 were included in the study. Main inclusion criteria were (i)IPSS Low and Int-1 MDS (ii) Hb<10g/dl or need for more than 2 red blood cell units of transfusions during the two months preceding the date of inclusion, (iii) serum EPO level<500mUI/ml (iv) de novo MDS, excluding therapy related cases.
Results: 62% (43% major and 20% minor) erythroid responses were seen, and median response duration was 20 months according to IWG 2000 criteria. Significantly higher response rates were observed with <10% blasts, Low and Int-1 IPSS, RBC transfusion independence, serum EPO level<200UI/l. Significantly longer response duration was associated with major response (IWG 2000 criteria), IPSS low-INT-1, blasts<5% and absence of multilineage dysplasia. Multivariate adjusted comparisons of survival between our cohort and the untreated MDS cohort used to design IPSS, showed similar rate of progression to AML in both cohorts, but significantly better overall survival in our cohort with five year- overall survival (OS) of 64% in the French-EPO cohort and of 39% in the IMRAW cohort. This survival benefit, in the French cohort, was restricted to patients who responded to rEPO. In multivariate analysis, rEPO treatment was independently associated with better survival.
Conclusions: Major prognostic factors of response to EPO±G were confirmed. Multilineage dysplasia was not associated with lower response rates, but with shorter response duration. Epoetin or DAR treatment may have a favourable survival impact in low-risk MDS.

Glial Cell Line-derived Neurotrophic Factor Family Artemin-Transcriptional Regulation, Neurite Outgrowth and Actin Polymerization in Mature Dorsal Root Ganglia Neurons

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Background: Neurotrophic factors are signaling molecules that regulate multiple aspects of the development of the nervous systems. The glial cell line-derived neurotrophic factor (GDNF) family includes artemin, persephin, and neurturin. GDNF was found to reverse the posttraumatic changes into nerve regeneration and neuropathic pain treatment. Artemin expression is observed in the adult brain and spinal cord, suggesting that artemin may play a role in the post-developmental stage. We performed a microarray analysis and real-time PCR experiment to investigate the patterns of gene expression underlying the effect of artemin on the mature DRG.
Methods: An *in vitro* isolated DRG model was used to study the effects of artemin on the adult rat neuronal system and investigate differentially regulated genes. For the DRG neuron culture, the ganglia were trypsinized. Total RNA was extracted and reverse-transcribed to double-stranded cDNA using an oligo-dT primer. The cDNA microarray containing a set of 5088 rat cDNA was used. The cultures were stained for vesicle endocytosis with FM 1-43 and for F-actin with phalloidin–Alexa568.
Results: 285 genes were differentially transcribed by artemin after 3 hour of treatment. A series of genes involved in the regulation of actin dynamics, including coronin, Myr 5, Wiskott-Aldrich syndrome protein interacting protein, cofilin, drebrin and dynamin were down-regulated by artemin, suggesting a previously undefined role in the regulation of actin polymerization and synaptic vesicle movement. Artemin also down-regulated the expression of genes related to cell adhesion and matrix assembly, including biglycan, plectin, nestin, neuronatin and the neuron-glia-CAM-related cell adhesion molecule, which is functionally relevant to neurite elongation in DRG neurons. Artemin resulted in increases in total neurite length and branching of the DRG neurons. Also artemin caused an increase of synaptic vesicle clustering. The inhibition of DNA methylation suppressed the artemin-dependent neurite growth.
Conclusions: 1) The mature DRG neurons showed some response to artemin, suggesting that they exhibited a developmental shift to the ligand as prenatal DRG neurons. 2) DNA methylation seems to provide a mechanism for artemin-dependent genetic regulation responsible for axonal growth.

Central glutamatergic dysfunction as an explanation of resistance and refractoriness in Obsessive-Compulsive Disorder

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About 50% of patients affected by Obsessive-compulsive Disorder (OCD) do not respond to pharmacological treatment with selective serotonin re-uptake inhibitors (SSRI) and with clomipramine, drugs considered the gold standard treatment for OCD. Resistant patients are those who participate in a trial with any first-line therapy and do not have a satisfactory response, while the refractory patients are those who do not respond appropriately to several treatments administered in an adequate manner. In patients who do not respond to the above treatments, therapeutical strategies include switching and augmentation. A number of studies have shown that switching strategies can determine an improvement of OCD symptoms in about 40% of refractory OCD patients. Other studies have demonstrated that clinical improvement can be obtained adding drugs acting on dopaminergic system (typical and atypical antipsychotic drugs). Recently, it has been shown that drugs acting on systems other than dopamine, in particular glutamatergic drugs can ameliorate clinical OCD symptoms. The glutamatergic system was included in the physiopathology of the OCD after the observation of an increase in the glutamate concentration in the caudate nucleus of children with OCD and normal glutamate levels after treatment with paroxetine. Increased glutamate levels in the LCR of OCD patients was also described. Drugs that modulate the glutamatergic system, as riluzole, d-cycloserine and memantine, were recently used for refractory OCD patients. Memantine blocks the N-methyl-d-aspartate (NMDA) receptor-associated ion channel, and acts as an uncompetitive, low-affinity, open-channel blocker that enters the receptor
channel preferentially when it is excessively open. The NMDA receptor is normally activated by the binding of glutamate, the major excitatory neurotransmitter in the central nervous system. It is believed that increased influx of calcium ions from the excessive activation of this channel may lead to excitotoxic damage to neurons in the brain. Neurotoxicity due to central glutamatergic hyperactivity may explain refractoriness to serotoninergic agents. There are still few data on the efficacy of the use of agents that affect the glutamatergic system of OCD patients however, the preliminary results seem to be promising.

Nitrendipine as a putative probe for CYP3A phenotyping

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Hepatic and small bowel CYP3A3/4 content and activity is known to vary 10-20-fold among individuals. Though such a wide interindividual variability has been explained by ethnic and/or diet differences, newer investigations make also genetic contribution evident. Our aim was to develop a simple method for sorting out the extreme metabolisers (both rapid and slow) of nitrendipine (metabolised also by CYP3A3/4) and to use this method in the bioequivalence study of two nitrendipine formulations. The peak plasma concentrations (c_{max}) of nitrendipine found after a single dose (20 mg) administration of nitrendipine tablet (Baypress) were evaluated. In a pilot study with 18 healthy volunteers, the c_{max} acquired values from 1,4 to 51,3 ng/ml (median 6,95 ng/ml). As the time to reach c_{max} (t_{max}) was usually 1 or 2 hours in the pilot study, we decided to include into pivotal bioequivalence study of two nitrendipine formulations only volunteers who showed: a) 1 or 2 hours after the "diagnostic 20 mg single dose" the nitrendipine plasma concentrations in the range 5-40 ng/ml (i.e. less then one order), and b)neither in 1 hour nor in 2 hour after administration these concentrations exceeding 40 ng/ml. Of 45 examined volunteers, 9 were extreme metabolisers (8 rapid and 1 slow). Remaining 36 healthy volunteers entered the bioequivalence study (open, randomized, cross-over) which proved bioequivalence of two compared oral tablet nitrendipine formulations conclusively (the 90% confidence intervals for AUCs and c_{max} lie within an interval of 0,80-1,25).

Retinoic Acid Regulates the Expression of the Anti-Apoptotic Protein PKCδVIII.

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Background: The human teratocarcinoma cell line, NT2 cells differentiate into hNT neurons upon treatment with retinoic acid (RA). This is a widely accepted model to investigate the expression of genes involved in neurogenesis, neuronal differentiation and early development of nervous system. Protein kinase C (PKC) δ plays an important role in the regulation of cell apoptosis. We have recently described a novel PKCδ isozyme- PKCδVIII that is expressed in human NT2 cells upon RA treatment. PKCδI and PKCδVIII are the alternatively spliced variants expressed in human cells.Expression of PKCδVIII peaks at day 1 of RA treatment and then declines by 7 days. RT-PCR analysis and sequencing data revealed that this isozyme is generated via utilization of a downstream alternate 5′ splice site of exon 10 which results in an insertion of 93 bp in the caspase-3 recognition sequence within the V3 domain. We have shown that PKCδVIII is resistant to caspase-3 cleavage and that PKCδVIII regulates *anti-apoptotic* effects in these cells.
Methods: RNA isolations of NT2 cells treated with retinoic acid were performed using RNA Bee (Tel Test, Inc.) RT-PCR was performed using primers that detect both PKCδ isoforms as well as primers specific for PKCδVIII.
Results: We have identified the nuclear serine-arginine rich splicing factor SC35 (i.e. SRp30b) which promotes the expression of PKCδVIII mRNA via utilization of the alternative 5′ splice site II on PKCδ exon 10. Western blot analysis demonstrates that the expression of SC35 increased with RA treatment concurrent with the increase in PKCδVIII expression. Overexpression of SC35 in NT2 cells promotes the expression of PKCδVIII. To further decipher the mechanism of alternative splice site selection we have designed and cloned a minigene which includes PKCδ exon 10 and its flanking introns in the pSPL3 splicing vector. We show that this minigene is responsive to retinoic acid. Further, co-transfection of SC35 with PKCδ minigene promotes selection of 5′ splice site II. Transfection of cells with SC35 siRNA along with PKCδ minigene results in a decline of PKCδVIII expression.
Conclusions: (1) PKCδVIII plays a role in development of nervous system (2) RA regulates expression of PKCδVIII via SC35.

(-)-α-Bisabolol – a Specific Ergosterol Biosynthesis Inhibitor ?

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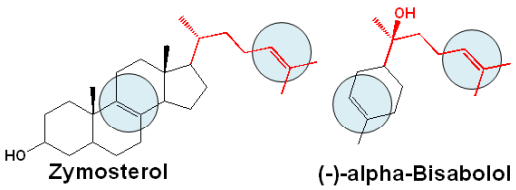
Background: At the level of zymosterol in the ergosterol biosynthesis all subsequent intermediates are solely produced by fungi. The recognition of a specifically acting inhibitor within this part of ergosterol biosynthesis would leed to a new class of antifungal drugs. To obtain information on the existence of compounds having both, structural similarities to fungi specific ergosterol precursors plus antifungal properties, previous findings in literature were analyzed in view of this aspect.
Methods: A chemical (sub)structure and MIC searchable computer database on antimicrobials (Amicbase) was used. In the data query antifungal compounds were searched, which include one of four different side chains types as they occur in the section from zymosterol to ergosterol.
Results: Among antifungals the side chain of zymosterol was most frequently found. Addition of oxygen and limiting to *Candida albicans* resulted in 19 different compounds, whose MIC increased with molecular volume (r = 0.77). Like zymosterol, (-)-α-bisabolol contains a cyclohexene ring plus a second double bond in similar interatomic distance. This may point to an inhibition of zymosterol conversion into fecosterol by (-)-α-bisabolol, which would offer the opportunity to disturb specifically fungal biochemistry.



(-)-α-Bisabolol from chamomile has antiinflammatory, wound-healing, anticancer, antispasmodic properties and inhibits fungi (3-100 µg/ml), gram-(+) (32-500), but less or not gram-(-) bacteria. Due to its low toxicity (monkeys tolerated oral 15 ml/kg bw) and the lack of reports on allergic reactions in its cosmetic use, the compound was taken to treat fungal and bacterial infections. Own case reports will be given.
Conclusions: The antifungal mechanism of (-)-α-bisabolol is worthwile for further investigation due to its safety and its unique pharmacological profile.

Differential roles of physiological and physicochemical parameters on low and variable bioavailability of Saquinavir- hurdles of effective drug treatment

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Background: Saquinavir (SQV), the first of the Human Immunodeficiency Virus (HIV) protease inhibitors to reach the market, remains one of the most widely prescribed agent which has markedly improved morbidity and mortality in HIV-infected patients. Inclusion of SQV in 'highly active anti-retroviral therapy' has substantially improved the clinical outcomes of AIDS patients. However, the complete therapeutic potential of this class of drugs is yet to be exploited due to number of limitations related to their poor and variable transport across important biological membranes, especially gastrointestinal tract. The pharmacokinetic profile of SQV being characterized by its low and variable bioavailability is primarily attributed to metabolism by cytochrome P-450 3A4. Moreover, there is increasing understanding that membrane transporters (P-gp, MRP-2) contribute significantly to the biopharmaceutical characteristics of SQV and this entire class of drugs. However, the relative contributions of these eliminating organs to the first-pass effect and the significance of incomplete absorption of SQV have not been explored.
Methods: In this study, our objective was to determine the various factors governing the bio-availability of SQV. In order to understand the contributions of i) first pass metabolism in gut and liver and ii) solubility and permeability of SQV across the lumen, towards its low bioavaibility, in-vitro, in-situ and in-vivo study results in rat model were compared. Though, the first-pass intestinal and hepatic metabolism has been shown to be a major determinant in the oral clearance of SQV, we found that physicochemical characteristics may also have an important role in determining the oral absorption and disposition properties of this drug. To best of our knowledge, we are the first to report a double-peak phenomenon in plasma concentration time profiles of SQV via oral administration. Although the double peaks in the plasma concentration-time profiles after p.o. doses could be attributed to a gradient expression of transporter proteins along the gastrointestinal tract, we hypothesize that the phenomenon is due to differential solubility profile of SQV across the intestine. In-vitro solubility data clearly indicates the pH dependent solubility profile of this drug. SQV has pKa values of 1.1 and 7.1, corresponding to the quinoline and octa-hydroisoquinoline nitrogens, respectively. A base pka of the drug just above pH 7.0, so the solubility would be expected to decrease significantly as the drug moves from duodenum (fasting pH about 6) to distal ileum (at about pH 7.8), and then increase again in cecum (at about pH 5). The time of the second peak (4-5 hours) coincides with the expected arrival time in rat cecum which was confirmed with intestinal motility test using charcoal.
To relate bioavailability to molecular transport characteristics, we, speculated that an efflux (counter transport) mechanism might contribute to the low and variable bioavailability of SQV. Single pass in-situ absorption method was employed for the determination of the permeability (P_{eff}) of SQV in various segments of rat intestine, viz. duodenum, jejunum ileum and colon. The data reveals that the P_{eff} of SQV through rat duodenum (2.91×10^{-5} cm/s) is higher than jejunum (1.86×10^{-5} cm/s) or ileum (2.11×10^{-5} cm/sec), which is in line with the higher extent of absorption of the drug in the duodenum. On the other hand, P_{eff} values of the drug in jejunum, ileum and colon were found almost similar. The results hence clearly suggest that SQV is a low permeable drug, apart from being poorly soluble at intestinal pH.
Conclusion: Because P-gp is found lower down the gut than is CYP3A, the exposure of SQV to P-gp is high because of its low solubility, and this could explain the very long period over which SQV is absorbed. Absorption, rather than elimination, controls the pharmacokinetics of SQV, and its very slowness is probably responsible for the low and variable kinetic profile of this drug. In conclusion, the bioavailability of SQV is controlled by a combination of solubility in the gut lumen, p-glycoprotein mediated efflux in the gut-wall, and first-pass intestinal and hepatic metabolism by CYP3A4. Given the differential and complex roles of physiological and physicochemical characteristics in SQV oral absorption, the optimization of AIDS boosting regimens requires careful consideration in order to avoid therapy limiting drug-drug transporter and enzyme interactions.

Discovery and Use of the Magic Bullets in Human Taeniosis (Niclosamide, Praziquantel)

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Background: Taeniosis/cysticercosis is a serious public health problem in several countries of Latin America, Africa and Asia. Approximately 2 500 000 people carry a *Taenia solium* tapeworm. Globally, conservative estimates calculate 50 000 deaths from neurocysticercosis every year.
Older taenicides: Several more or less toxic natural remedies, such as male fern extract, Kosso flowers, areca nuts, pomegranates have been used for centuries. In early 20th century in Germany among 22 000 cases of taeniosis treated with male fern extract 18 patients died and 71 others have lost vision. Several synthetic drugs were tried as taenicides in 20th century e.g. thymol (1912), carbon tetrachloride (1931), hexylresorcinol (1932), mepacrine (1947), dichlorophen (1956), bithionol (1962), paromomycine (1967) and mebendazole (1975). In 1950s my patients with *T.saginata* taeniosis were treated with pumpkin seeds (cure rate 65% among 163 treated), atabrine (respectively 42%, 44), acranil (88%, 89) or metallic tin compounds (88%, 226). Many of these drugs were unsafe or poorly tolerated, although some were rather effective.
Modern taenicides: The first magic bullet was - niclosamide, introduced in 1959. As a barely absorbed substance it is safe and well tolerated. However, it's early original version (and still some generic products) has lost the efficacy during storage due to polymerization of its active particles. The efficacy of a single dose of niclosamide in human taeniosis is about 85%. Since 1972 niclosamide has been gradually replaced by the second magic bullet - praziquantel, being more stable taenicide, more efficacious (95%) and much cheaper (10 US cents for a dose). It has been used widely in the control of schistosomoses. Due to autoinfection more than 10% of *T.solium* tapeworm carriers develop neurocysticercosis. Therefore, the use of praziquantel in control of taeniosis is questioned, as this drug may damage existing brain cysticerci and change asymptomatic neurocysticercosis into a symptomatic one. The problem is partly solved by a reduction of the dose of praziquantel in taeniosis to 5-10mg/kg b.w. in a single dose. Still some uncertainty exists whether it is worth to risk such a rare side effect at a mass-treatment in *T.solium* endemic areas.
Conclusions:
1)There is still a place for another magic bullet in taeniosis/cysticercosis.
2)Nitazoxanide, a broad spectrum antiparasitic drug, is waiting in a row.

Metallic gold reduce TNFα expression, oxidative DNA damage and pro-apoptotic signals in brain injured mice.

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Background: Traumatic brain injury (TBI) continues to represent a major global health problem, and represents a leading cause of morbidity and mortality in young individuals. There is therefore an imperative need for neuroprotective treatments limiting the neurologic impairment following such injury. Gold compounds have been used for treating the inflammatory condition *rheumatoid arthritis* for over 50 years. Due to the well-established anti-inflammatory effects of gold compounds in the treatment of arthritis and the known role of inflammation in fueling cell death in the injured brain, we investigated the potential of metallic gold in alleviating inflammation, oxidative stress and apoptotic markers of cell death following experimental traumatic brain injury in mice.
Methods: C57BL6 mice were subjected to a unilateral traumatic cryo(freeze)-lesion with concomitant injection of 25-45 µm gold particles suspended in hyaluronic acid near the lesion. Placebo-treated mice served as controls. Immunohistochemical markers of inflammation, oxidative stress and apoptosis were then compared in the gold- and placebo-treated animals at 1 and 2 weeks post-lesion.
Results: The study revealed a statistically significant decrease in tumor necrosis factor alpha (TNFα) and oxidative stress (as judged by immunohistochemical staining for 8-oxoguanine) in gold-treated animals, as compared to controls. Moreover, gold-treatment resulted in a statistically significant decrease in the release of cytochrome c from the mitochondria and in the activation of caspase-3.
Conclusion: We have previously shown that intracerebral deposition of metallic gold liberates gold ions that reduce microglial activation and neuronal apoptosis, while increasing reactive astrogliosis and neuronal stem cell response following focal brain injury. In this study we showed that bio-liberated gold ions result in marked anti-inflammatory, anti-oxidative and anti-apoptotic responses in the injured brain, and could have clinical potential for treating traumatic brain injuries. The findings thus support the role of metallic gold as a neuroprotective compound. We further hypothesize that this local gold implant application results in negligible systemic disseminations of gold ions, hereby limiting the risk of severe adverse effects, such as nephrotoxicity. We therefore propose that metallic gold implants may be useful as an enhancer of neuronal protection and regeneration following both TBI and perhaps in many other neurodegenerative conditions.

New Calcipotriol Analogs, their Toxicity and Antitumor Activity in vivo in Comparison to the Affinity with VDR and DBP

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Background: Calcitriol has been proven to be a potent antiproliferative agent against various normal and neoplastic cells. Moreover, calcitriol and other vitamin D analogs revealed the ability to induce differentiation of many human cancer cells. Biological activity of these compounds is mediated by the nuclear vitamin D receptor (*n*VDR). Calcitriol is carried with plasma Vitamin D Binding Protein (DBP). The affinity with DBP could be responsible for the toxicity and bioavailability. Such biological properties suggest the potential therapeutic application for these agents, including antitumor therapy. Two of the promising calcipotriol analogs: PRI-2202, PRI-2205 and the paternal compound have been the objects of our intensive studies.
Methods: In this work, we present results of the affinity of different analogs of vitamin D with VDR or with DBP using Molecular Operating Environment (MOE) program. Furthermore, the toxicity, calcemic activity and antitumor activity of these analogs in the LLC mice tumor model were tested.
Results: PRI-2205 analog exhibited the highest affinity with VDR and DBP. PRI-2205 analog exhibitad both the low toxicity and calcemic activity and the highest antitumor activity with comparison to other derivatives.
Conclusion:
1.Based on these results, we could formulate the hypothesis about the positive correlation between the antitumor activity of new calcioptriol analog and its affinity with VDR.
2.Its lower toxicity and calcemic activity seems to be correlated with its higher affinity with DBP.
The authors are grateful to Chemical Computing Group Inc for making the MOE program available for free testing work.

Modifying cytoplasmic protein complexome involved in energy metabolism as a tratégy of *Escherichia coli* against ceftriaxone

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Background: Recently, altered proteins or proteomes in response to antibiotic resistance are systematically investigated, but information regarding to a protein complexome in the resistance is not available. Aims: 1) To establish a cytoplasmic protein complexome involved in ceftriaxone (CRO) resistance by combined 2-D native/SDS PAGE and protein-protein interaction approaches, 2) To investigate functional characteristics of the complexome using genetic modified strains of the gene deletion of the proteins involved.
Methods: Combined 2-D native/SDS PAGE and Co-IP, far-Western blotting or His-tag pull down assays were utilized for the cytoplasmic protein complexome of *E. coli* involved in CRO resistance. Antimicrobial susceptibility and carbohydrate metabolism of the complexome was investigated by minimun inhibitory concentrations (MIC) and survival capability assays of genetic modified strains with gene deletion of the proteins.
Results: MalP, AtpD, PflB, LysS, MalE and CysK were found to be down-regulated, and SucC and SucD were up-regulated in the CRO-resistant *E. coli* strain. They respectively belonged to seven protein complexes, MalP homodimer, AtpD homodimer, PflB-TnaA, LysS homodimer, MalE-TalB, CysK-SodB and SucC-SucD. Most of them were involved in carbohydrate metabolism. MalP homodimer, SucC-SucD, LysS homodimer and CysK-SodB may play more important roles in the control of CRO resistance than other complexes identified. On the other hand, CRO-R and these mutants grew normally as the same as CRO-R-O in LB medium but most of them showed growth-combating in M9 medium with fructose, D-mannitol, maltose, glycerol or glucose. Importantly, up-regulated SucC and SucD in 2-DE gels respectively played negative and positive role in regulation of TCA circle and CRO resistance. All complexes but SucD-SucC were contributed to down-regulated the classical carbohydrate pathways as a strategy against CRO.
Conclusions: Cytoplasmic protein complexome involved in CRO resistance of E. coli was achieved by proteomic methodologies based on combined 2-D native/SDS PAGE and Co-IP, far-Western blotting or His-tag pull down assays. Eight differential expressed proteins and three new protein complexes, TnaA-PflB, MalE-TalB and CysK-SodB were identified from the cytoplasmic fraction. Among these proteins, down-regulated of MalP, AtpD and PflB, up-regulated of SucC-SucD, which involved in modification of energy production and conversion, may play key roles related to resistance according to the antimicrobial susceptibility and carbohydrate metabolism analyses using genetic modified strains with gene deletion of the proteins. These findings provide new insights into the mechanisms of CRO resistance, and these key proteins can be the targets for development novel antibiotic compounds. In our knowledge, this is the first report on functionally altered complexome and energy metabolism modification to antibiotic-resistant bacteria. This work was sponsored by grants from National Basic Research Program of China (2006CB101807) and "863" project (2006AA09Z43Z).

Innovative Nanopharmaceutical Strategies for Neuronal Survival and Regeneration After Traumatic Brain Injury

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Background: Metallothionein (MT) is a multipurpose cytoprotectant important for host defense responses, immunoregulation, and cellular survival. We have explored MT functions in the injured brain *in vivo* and tested the pharmaceutical effects MT. The aim is to develop treatment strategies for neurological patients.
Methods: This study applied genetically modified mice with MT deficiency or overexpression along wildtypes. The mice were subjected to a focal brain injury and in the following weeks, they received i.p. injection of exogenous MT protein or placebo. Animals were sacrificed at different time points and brains were removed for histopathology, molecular analyses and global gene profiling.
Results: In injured brain, MT inhibits inflammation by reducing macrophages, T lymphocytes and formation of proinflammatory interleukins, tumor necrosis factor-alpha, matrix metalloproteinases, reactive oxygen species and proapoptotic factors. MT enhances neuronal survival and brain repair processes including angiogenesis and neuroregeneration. The latter is due to activation and recruitment of resident neural stem cells and neuroglial precursor cells. Both endogenous and exogenous MT had neuroprotective actions. Peripherally injected MT is well tolerated and is detected in the brain within 1 h after i.p. administration. This transport of MT from peripheral tissues into the brain is likely facilitated by blood-brain barrier leakage caused by the traumatic injury, which transiently disrupts integrity and permeability of the blood-brain barrier. The intercellular MT signaling is mediated in part by megalin, a multiligand endocytotic receptor and by lipoprotein receptor-related protein-1 (LRP). The intracellular signaling of MT involves kinases, phosphorylation and dephosphorylation-mechanisms, G-protein coupled receptors, and zinc ion regulation, although the precise mechanisms downstream of MT remain to be fully established.
Conclusions: The data demonstrate the importance of MT for neuroprotective and regenerative responses following traumatic brain injury. MT is an antiinflammatory and antiapoptotic factor, which promotes brain tissue repair and functional recovery. MT may provide a novel pharmaceutical strategy against brain trauma.

Adverse Reactions of Titanium-based Chemotherapeutic Agents on Male Reproductive Organs

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Background: Although the treatment efficacy of some titanium compounds as cytostatic agents on cancers is known, some side effects must be better estimated. Our previous studies have shown that titanocene dichloride, a metal-based antineoplastic complex, disrupted the blood-testis barrier in mice. Aims: 1) To compare the effects of two concentrations of titanocene dichloride on testis. 2) To evaluate sperm quality. 3) To estimate possible recovery of the lesions.
Methods: Male mice (2 months old) were subcutaneously administered with 0.12mg/g and 0.15mg/g/body weight of titanocene dichloride *per se*, and sacrificed after 72h. Other groups were injected similarly and kept for 35 days without any treatment. Controls were also considered. Animals were sacrificed for organs removal. Testes were fixed in Bouin´s solution and prepared for histology. Caudal epididymal fluid was collected for sperm quality analysis using standard procedures. Observations and micrographs were made using a light Nikon AFX-Dx microscope.
Results: A surviving rate of 100% was noted through the present study. However, histopathological studies have shown a considerable loss of germ cells after 72h, derangement of cellular organization within spermiogenesis of mice treated with the lowest dose. In comparison, the other group (0.15mg/g/body weight) showed more evidenced lesions. The sperm analysis from these groups of mice revealed a wide range of abnormalities in the concentration, motility, vitality, and morphology. After one cycle of spermatogenesis, a partial recovery of the lesions was denoted in the testis and seminal fluid from the group administered with 0.12mg/g of titanocene dichloride. However, no reversible effects were detected in the highest dosed group of animals. Spermiogenesis, and sperm quality were deeply affected.
Conclusions: 1) These results confirm that titanocene dichloride impairs at least spermiogenesis. 2) The persisting abnormalities observed after the recovery period suggest that this fact may compromise male fertility.
Future studies must address the biochemical and molecular approaches of the blood-testis barrier, for the understanding of the pathophysiological mechanisms leading to the testis and epididymis alterations.

The differential dose-dependence for pro-angiogenic and cytotoxical effects of new anticancer plant agent within different nanocarrier formulations

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New drugs for cancer have been tested with a promising success. Molecular plant components were far ahead aimed into the angiogenesis and anti-angiogenesis pathways. Those molecules were able to destroy the mitochondria of cancer cells, what made scientists focus on the cancer cells themselves, not considering the destruction of the blood feeding system to eliminate the tumor. Another point revealed as a difficulty was the poor capacity of these drugs to be administrated into an in vivo analyzing system, to prove other mechanisms of promoting the cancer cells death. The present paper deals with the construction of a nanocarrier system, in which a molecule of an anticancer plant agent was included. The effects of these new constructs were tested into the Chorioallantoic Membrane (CAM) in the chicken embryo model. A14 stimulates human umbilical vein endothelial cell (HUVEC) proliferation in the 1-10 micromolar range, and promoted cell death in the 1-10 milimolar range. It was also toxic to Murine melanoma B16F10 in 1-10 milimolar concentrations, even as proliferative effects were not observed. In vivo pro-angiogenic effect was observed in CAM, which presented increased number of blood vessel ramifications and capillary budding. The new vessels presented leakier and less organized than those from control membranes. When molecular drug carriers were employed, the pro-angiogenic A14 effects could be observed at nanomolar concentrations, either in cell cultures or in the CAM model. Accordingly, treatment with A14 or its formulation dramatically reduced the number and size of the disseminated B16F10 murine melanoma tumors in the CAMs of live eggs. It is proposed that not only direct toxicity against B16F10 cell, but also the drug effects upon angiogenesis were relevant to both decreasing tumor growth and inhibiting metastatic dissemination in the *in vivo* model of neoplasia.

Chemical agents in root canal therapy. Key of the success

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Background: The ideal irrigating solution or combinations of irrigating solutions at appropriate concentrations and pH, or the time of application of each one to achieve success in the cleaning and disinfection of the root canal is not known. Aims: To determine which irrigating agent cleans better the root canal after rotatory and manual instrumentation and which one is a decalcifying agent of the root canal dentin wall eventually.
Methods: 80 human teeth were randomly divided into 8 groups. 4 groups were prepared with hand instrumentation and other 4 with rotary. The irrigating solutions were 2.5% NaOCl; 15% citric acid *plus* 2.5% NaOCl; 15% EDTA *plus* 2.5% NaOCl; and 5% orthophosphoric acid *plus* 2.5% NaOCl. Canal walls were observed with SEM, and photomicrographs were taken in three thirds to evaluate the cleaning ability. In order to study the decalcifying effect, two slices were cut from the cervical third of the root of 10 human incisors. Each slice was sectioned into 2 equal parts. Specimens were assigned to one of four groups (n=10) for immersion in 15% EDTA, 15% citric acid, 5% phosphoric acid or 2.5% NaOCl, during 5, 10, and 15 min. The concentration of Ca^{2+} was measured by AAS.
Results:

Cleaning ability	2.5% NaOCl	2.5% NaOCl + 15% citric acid	2.5% NaOCl + 15% EDTA	2.5% NaOCl + 5% orthophosphoric
APICAL				
Manual	3.91±0.28	2.80±0.85	2.15±0.80	2.74±0.67
Rotary	4.57±0.40	2.07±0.92	2.30±0.96	2.91±1.03
MIDDLE				
Manual	3.91±0.26	2.06±0.91	1.44±0.55	2.23±0.65
Rotary	4.63±0.38	1.72±0.70	1.78±0.84	2.29±0.99
CORONAL				
Manual	3.85±0.27	1.13±0.32	1.65±0.87	1.79±0.65
Rotary	4.74±0.36	1.19±0.43	1.35±0.44	2.30±0.90

At 5, 10, 15 min 15% EDTA (0.085±0.029; 0.094±0.028; 0.098±0.028) and 15% citric acid (0.075±0.019; 0.093±0.024; 0.099±0.027) extracted the largest amount calcium. The most rapid decalcification rate was with 15% EDTA. NaOCl extracted negligible calcium.
Conclusions: Acid solutions with 2.5% NaOCl were more effective than 2.5% NaOCl in the root canal cleaning with both techniques of instrumentation. It is the same for decalcification, acid solutions produce decalcification of root dentin, with most calcium extracted during the first 5 min of action.

Increased Expression of Tumor-Specific Cyclin B1 Sensitizes Prostate Cancer Cells to Apoptosis Induced by Anti-Mitotic Drugs

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Chemotherapeutic drugs ideally should take advantage of the differences between transformed and normal cells and induce apoptosis only in cancer cells. One such difference may be the overexpression of cyclin B1 protein in cancer cells, which is required for the proper progression through mitosis and is considered to be a tumor-specific antigen. We showed that treatment of human prostate cancer cells with the promising antimitotic drugs paclitaxel, docetaxel, or 2-methoxyestradiol (2ME2, a metabolite of estradiol) results in a rapid accumulation of cyclin B1 protein and an increase in cyclin B1 kinase activity, followed by induction of apoptotic cell death. Inhibition of cyclin B1 kinase lowers apoptosis induced by paclitaxel, docetaxel, and 2ME2. We hypothesize that a cancer-specific mechanism whereby these antimitotic drugs may exert anti-prostate cancer activity is the deregulated activation of cyclin B1 kinase during the cell cycle, leading to the induction of apoptotic cell death. Our results in human prostate cancer cells established a positive correlation between cyclin B1 protein and apoptosis induced by chemotherapy. There is minimal cyclin B1 and induction of apoptosis by chemotherapy in non-transformed cells. Stable overexpression of cyclin B1 in human LNCaP prostate cancer cells increases sensitivity to apoptosis, possibly by decreasing anti-apoptotic Bcl-2 and increasing pro-apoptotic p53. Experiments using siRNA and a dominant negative mutant of CDK1 to lower cyclin B1 protein and kinase activity in LNCaP cells showed a decrease in apoptosis.
We then determined whether the combination of docetaxel and 2ME2 can increase apoptosis utilizing human prostate cancer cell lines and the FG/Tag (fetal globin promoter linked to SV40 T antigen) transgenic mouse model of androgen-independent prostate cancer (AI-PC). Cell growth, flow cytometry, and apoptosis assays demonstrated that the combination of low doses of 2-ME (0.5 mM) and docetaxel (0.1 nM) can inhibit growth, increase G2/M cell cycle block, and induce apoptosis more significantly than either drug alone in a variety of human prostate cancer cell lines. In the FG/Tag mice, a low dose combination of docetaxel and 2ME2 (2.5 + 75 mg/kg) significantly inhibited primary prostate tumors by 42% ($P<0.02$), despite each dose of the drug by itself having no effect. Reduction of primary prostate tumor weights required doses of docetaxel and 2ME2 that increased mitotic cell cycle arrest and increased apoptosis, as shown by flow cytometry and immunohistochemistry. In contrast, inhibition of proliferation or angiogenesis without an effect on apoptosis did not reduce the weights of primary prostate tumors. Docetaxel and 2ME2-mediated increase in mitotic block occurred only in cyclin B1 overexpressing prostate tumors and not in normal tissues (negative for cyclin B1).
Conclusion: The ability of the antimitotic drugs paclitaxel, docetaxel, and 2ME2 to induce apoptotic cell death in AI-PC cells will probably one of the mechanisms required for an improved patient survival benefit. Our results suggest that despite its association with transformed cells, higher levels of cyclin B1 protein in prostate cancer may be a good prognostic marker for chemotherapy induced apoptosis.

Status of BCG Vaccine at the Beginning of the 21st Century

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Background: Since tuberculosis (TB) is declared global emergency by the World Health Organization, TB vaccine development is making rapid progress. Virtually hundreds of candidate vaccine antigens are currently passing through different phases of preclinical research and several new vaccine products are now in trials' phases in humans to achieve the formulation of a superior TB vaccine. It was in 1921 when *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) vaccine was administered for the first time, with success, to an infant born to a mother who died for TB and brought up by a grandmother suffering for TB. BCG vaccine is almost 90-year old now and the world still does not have anti-TB vaccine which would be better or with less adverse reactions than BCG is. The vaccine is able to protect newborns and infants from the most serious forms of TB – TB meningoenceplalitis and to prevent death of TB. Despite a number of shortcomings, BCG is likely to be administered for at least the next 5-10 years in protection against TB. Also known as a powerful immune modulator, it is used in many ways to treat other conditions.

The aim of this presentation was to **review** the current status BCG vaccine, including the general view on the immunization policies, which differ among countries, to explain reasons for its variable efficacy and effectiveness**,** duration of protection against tuberculosis, cost/effectiveness studies and general plans on vaccination compared to the World Health Organization recommendations and national legislation. Particular issues like BCG vaccination in HIV-infected persons and immunization of TB high risk groups will also be presented together with cost-benefit models, which explored the utility of BCG in health care settings in the countries where BCG vaccine was not mandatory. The results of this latter encouraged a return to a policy of selective use of BCG among health care professionals**.** The International Union against Tuberculosis and Lung Disease suggested the criteria for the countries which would shift from routine BCG to a selective vaccination or its discontinuation. They are based on fulfilled TB epidemiological conditions in a particular setting.

High-dose Levofloxacin to Reduce Duration of Therapy and Slow Emergence of Resistance

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Background: The quinolone class of antibacterials was introduced more than 40 years ago with the accidental discovery of nalidixic acid as a by-product of the synthesis of the antimalarial compound, chloroquine. Naldixic acid had a narrow spectrum of antibacterial activity, poor absorption, and significant adverse events.
Methods: Extensive pharmacologic and clinical development of the quinolone class eventually led to the discovery and development of the fluoroquinolone, levofloxacin.
Results: With a broad spectrum of activity that targeted the most relevant respiratory pathogens; excellent pharmacokinetics including nearly complete absorption from the GI tract, broad tissue penetration, high serum concentrations and half –life suitable for once-daily dosing; levofloxacin was broadly adopted as the first true respiratory fluoroquinolone.
Conclusions: Levofloxacin remains a potent and relevant antibacterial therapy today. Additional pharmacodynamic development of a high dose, short course treatment regimen of levofloxacin achieved the specific objectives of enhanced bacterial killing, reduced potential for emergence of bacterial killing, and has pioneered a paradigm shift towards short-course therapy of respiratory infections.

Detemir Insulin in Type 1 and Type 2 Diabetes

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We have learned from our experience with the first long-acting insulin analogue, Insulin Glargine (Lantus) that a continuous dose of insulin is beneficial. This insulin relies on a less soluble insulin molecule, which becomes soluble when it interfaces with neutral pH. This basal insulin forms a depot subcutaneously and the insulin is slowly released. A revolutionary concept in insulin therapy was introduced in March 2006. Insulin Detemir (Levemir) is a clear, colorless, aqueous, neutral sterile solution, which protracts and maintains duration of action of up to 24 hours. Insulin Detemir (Levemir) is the first insulin that has a fatty acid side chain, which binds with albumin and protracts in a manner that provides a decrease in intra-patient as well as intter-patient variability.
Clinical Pharmacology: Recombinant DNA technology has led to the development of novel insulin molecules with absorption and biological activity profiles that are similar to physiological insulin. The amino acids threonine in position B30 has been omitted, and a C14 fatty acid chain has been attached to the amino acid B29.
Mechanism of action: Insulin Detemir exerts its action by binding to insulin receptors, lowering blood glucose by facilitating cellular uptake of glucose into skeletal muscle and fat. Insulin Detemir acts like all insulins inhibiting output of glucose from the liver. Insulin inhibits lipolysys, inhibits proteolysis and enhances protein synthesis.
Pharmacokinetics/Pharmacodynamics: Insulin Detemir (Levemir) has a relatively flat action profile. Glucose clamp studies have demonstrated efficacy at doses from 0.1 Units/kg with a mean duration of action of 5.7 hours to 23.2 hours with doses of 0.4 Units/kg. The sampling ended at 24 hours and hence it is felt to have efficacy up to 24 hours when the clamp study was discontinued. A slow systemic absorption of insulin Detemir molecules from the injection site is due to the strong self-association of the drug molecules and albumin binding.
Distribution: Likewise, due the albumin binding there is a slower, less variable distribution of the insulin Detemir molecules to peripheral target tissues. Since detemir is 98% albumin bound, binding studies were performed and found no clinically relevant interactions between insulin detemir and fatty acids or other protein-bound drugs. These insulin detemir molecules aggregate in a continuum from hexamer to di-hexamer and it is the monomer that binds with the receptor exerting its action. We report on the use of insulin detemir in Type 1 and Type 2 diabetes. Since insulin detemir (Levemir) can be given once or twice daily, lasts 16-24 hours, has a relatively flat action profile and does not require resuspension before injection it provides a greater flexibility of dosing regimens for the patients to follow. It is available in the ever popular FlexPen as well as vial. It should not be mixed with other insulins in the same syringe. It can be initiated on a Unit per kg basis as in Type 1 Diabetes. Generally, a unit per unit conversion is made comparing it with glargine, and 80% of the total NPH dose. Basal/Bolus therapy is usually initiated by spitting the insulins as half with 50% going to basal and 50% going to meal time insulin divided traditionally into three based on meal times. Initiating Levemir in Type 2 Diabetes is simpler. Generally patients are begun at 0.2 units/kg or 10 units daily with weekly titration. Some advocate sampling the average of three fasting blood sugars and increase the dose by 1 unit for every 40mg/dL blood glucose beginning at 180mg'dL. Through their own experience with basal insulin in partnership with their provider patients learn the merits of achieving A1C goals with a less risk of hypoglycemia. The greater flexibility of dosing and lower risk of hypoglycemia makes the initiation of this new insulin with greater ease.
Recommendations and conclusions: Insulin Detemir is an extraordinary basal insulin that will provide patients with less variability and hence less hypoglycemia. The concern of weight gain to achieve tighter control of blood glucose levels has always been a concern. This unique insulin delivery to the peripheral tissues will achieve normalization of glucose levels with no weight gain or possibly weight loss. Patients will accept insulin therapy more readily with the expectation of less hypoglycemia and the potential weight loss.

Tetracycline Biosynthesis: How to Overcome Nature's Potential in Developing Novel Anti-infectives?

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Background: Tetracyclines (TCs) are large group of medically-important antibiotics with a common basic structure of four linearly fused six-membered rings. They are produced by several genera from the order *Actinobacteria*; some examples are tetracycline, chlortetracycline, oxytetracycine, and demethylchlortetracycline, synthesized by the type II polyketide synthase (PKS) multi-enzyme complexes. A number of potent antibacterial TCs have been generated using a semi-synthetic approach such as minocycline, doxycycine and novel tigecyclin. TCs act at the ribosomal level interfere bacterial protein synthesis. They were amongst the first broad-spectrum antibiotics and their intensive use led to widespread microbial resistance. A small group of tetracycline analogs has recently been identified that do not target bacterial ribosome. Instead, they have bactericidal rather than bacteriostatic activity, and are active even against the tetracycline-resistant strains.
Methods: The objective of our work was to clone, sequence and characterize a novel gene cluster encoding an unusual TC antibiotic.
Results: Consistently with the chemical structure of the antibiotic, the cluster encodes genes for a typical minimal PKS (KSα, KSβ and ACP), three genes involved in the cyclisation/aromatisation process, methyltransferases, one aminotransferase, oxygenases, a ketoreductase, an acyl-CoA ligase, a drug resistance transporter and a transcriptional regulator were identified. The application of biosynthetic engineering methods in combination with well-established semi-synthetic approaches will be presented.
Conclusions: The unusual structure of this tetracycline analog provides an opportunity for development of new tetracycline molecules.

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Prokinetic Effect Of Erythromycin: The Benefit Of A Common Side Effect

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Background: Erythromycin was firstly introduced in the clinical practice in 1950 as antibiotic. The prokinetic activity of erythromycin was discovered in the 1980's as an adverse effect of the drug. Since then erythromycin has been used in patients with chronic functional pseudo-obstruction, gastro-oesophageal reflux post-operative intestinal dysmotility, gastroparesis secondary to diabetes, hyperglycaemia and scleroderma and after surgical vagotomy. Its use as a prokinetic agent has also been extended to preterm infants with gastrointestinal dysmotility and feeding intolerance and more recently within the context of critically ill patients. It is effective as a prokinetic drug on gastroparesis related to acute pain-related stress and when given prior to elective surgery has been shown to improve gastric motility. However, the aim of the present study was to investigate whether Erythromycin accelerates the delayed gastric emptying of solids and hypertonic liquids induced by hyperglycemic conditions.
Methods: Twelve healthy subjects ate standard radiolabelled solid and hypertonic liquid meals. Gastric emptying was measured by scintigraphy during normoglycemia (5-8.9 mmol/L glucose) and hyperglycemia induced by intravenous glucose (16-19 mmol/L glucose) after administration of placebo or 200 mg of erythromycin intravenously. Emptying was measured randomly on 4 different days.
Results: Administration of erythromycin during normoglycemia or induced hyperglycemia compared with placebo, accelerated the gastric emptying of the solid meal while gastric emptying of the hypertonic liquid was reduced. Eythromycin versus placebo significantly reduced the lag-phase duration, The lag-phase duration was significantly increased (17.5 +/- 5.5 min, and 7.2 +/- 4.5 min vs 10.5 +/- 3.4 min, and 3.5 +/- 2.5 min, respectively, $P < 0.0001$) as were the overall T1/2 (gastric emptying time of the half meal) (52.5 +/- 13 min and 24.5 +/- 5.5 min vs 42 +/- 10.5 min, and 16 +/- 6 min, respectively, $P < 0.0001$) and the percentage of liquid meal retained in the stomach at 60 and 100 min postprandially ($P < 0.001$). Gastric emptying of the half meal, and the percentage of meal retained in the stomach 120 min postprandially
Conclusions: The erythromycin-induced effect on gastric emptying of solids and hypertonic liquids is related to the plasma glucose level. Erythromycin accelerates gastric emptying rate of both solids and hypertonic liquids in both conditions, either in normoglycaemia or hyperglycaemia

Combinatorial Nanobiotechnology – A Paradigm Shift in Chemistry and Material Science

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Background: The more than 20-year evolution of phage display has dramatically affected the potential of this technique amid other bioengineering methods. The marriage of combinatorial chemistry and biological selection has been very powerful changing the methodology of biochemical research by allowing selection simultaneously among billions of genetic species in one test tube. Genetically driven phage nanobiotechnology has allowed development of libraries of diverse nanostructures expressed on the phage surface providing a rich resource of diagnostic, detection and pharmaceutical probes.
Methods: Phage engineering, which is based on natural mechanisms of selection, amplification and self-assembly, allows directed nano-fabrication of bioselective materials with possible applications in gene/drug-delivery, biosensors, nanoelectronics, biosorbents, and other areas of medicine, technology, and environmental monitoring. In particular, landscape phage expressing tumor-specific peptides fused to all copies of the major coat protein pVIII can be converted easily into gene-encapsulating particles or drug-loaded vesicles that acquire the ability to recognize the same receptors, cells, tissues and organs that have been used for selection of the precisely targeted phage. The fusion major coat protein constitutes 98% of the total protein mass of the virion — a purity hardly attainable in normal synthetic and bioengineering procedures. As a normal intestinal parasite, phage and its components are not toxic and have already been tested for safety in preclinical and clinical trials. All these unique characteristics of phage commend it very well as a very promising nanomaterial for a variety of medical and technical applications. To illustrate the concept, the author will present the data obtained in his research group and collaborative research.
Results: This presentation focuses on the progress made in the development of these new nanomaterials and discusses the prospects of using phage as a bioselectable molecular recognition interface in medical and technical devices based on the experience of the author in this area.
Conclusions: Phage display evolved into a discipline of material science presenting phage not only as an instrument for peptide and antibody discovery, but also as a prospective nanomaterial that can be easily tailored using routine genetic engineering manipulations. This merge of phage display technologies with nanotechnology during the last several years is very promising and has already shown its vitality and productivity contributing vigorously to different areas of medicine and technology, such as medical diagnostics and monitoring, molecular imaging, targeted drug and gene delivery, vaccine development, as well as bone and tissue repair.

Magic bullet or magic diet: The need for further understanding in sport performance enhancements

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Background: Translational nutrition embodies the use of dietary components to effect a therapeutic end point commonly associated with medicinal intervention. For example, recent advances have been made in the study of dietary chelators for treating neurodegenerative diseases via dissolution of plaques and by formation of anti-oxidnat enzyme mimetics. Further work has posited dietary components as regulators of gene product formation to enhance key protective enzymes such as superoxide dismutase. However, many scienticfic studies have focussed on individual components of diet (e.g. anti-oxidants) with little or scant regard for the entire profile of a foodstuff. In this era of multiple advance in integrative translation nutrition, further emphasis on the informed decision making regarding function nutrition is warranted. The aim of this study is to deconvolute decision making rationale versus practice for functional nutrition use in elite athletes.
Methods: The 'UK Sport 2005 Drug Free Survey' data (n = 874 adult and n = 403 youth elite athletes) were re-analysed using association [chi-square] and 'strength of association' tests [phi] to show the proportion of informed choices and to unveil incongruencies between self-reported supplement use and the underlying motives.
Results: Participants reported supplement use for performance enhancing and health maintenance reasons. Of the 30 possible associations between supplements and reasons, 11 were predictable in the first and 10 in the latter category from literature precedents, In the adult athlete population, only 8/11 were evidenced and these were not strong (phi < .7). The best associations were for the ability to train longer and maintaining strength with creatine and whey protein. Associations with health maintenance motives were found in 8/10 test pairs, however only weak associations exist. Of these, 4 were associated with avoidance of sickness (iron, multivitamin, vitamin C and Echinacea). Similar results were found among young elite athletes, where supplements were taken for performance enhancing reasons. No agreement was observed between athletes' rationale and behaviour except for creatine.
Conclusions: These results suggest that a lack of understanding exists in supplement use. There is an urgent need to provide accurate information which will help athletes make informed choices about the use of supplements.

MRI monitoring of blood brain barrier alterations in inflammatory lesions of Multiple Sclerosis: Tools to evaluate disease activity, efficacy of treatments and to develop new therapeutic strategies

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Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). Since 1999 we have pioneered a new approach of cellular and molecular imaging in vivo in application to MS. In an experimental animal model of MS we have monitored in vivo by Magnetic Resonance Imaging (MRI) the infiltration of the CNS by inflammatory blood cells (monocytes, T cells) labelled with iron nanoparticles as contrast agent in comparison to Gadolinium enhanced rupture of the blood brain barrier (BBB), and we have transferred this innovative approach for the first time to clinical research in MS patients. In experimental work and MS, this new approach has been validated as helpful tool to characterize acute inflammation and active inflammatory lesions, to predict the severity of disease development and to monitor the efficacy of immunomodulatory treatment strategies.

Ongoing experimental work aims to identify molecular alterations at the BBB that occur at early stages in EAE rats, by phage display screening in CNS characterized by macrophage infiltrates with MRI. We further develop a strategy by using the MRI cell marker and peptide-ligand binding approach to inflammatory lesions sites for transport of therapeutic compounds into the lesion sites and their local liberation under the control of MRI.

Is there a magic bullet in antiviral therapy?

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Antiviral therapy has been highly specific and tailored typically to treat one strain of a virus, but has also been problematic because viruses escape targeted therapy by mutation. This has been of increasing importance for example in the treatment of globally spreading viral infections like the HIV-infection, hepatitis C and other viral infections. An antiviral treatment that would fulfill the requirements of a magic bullet would however need to have a different type of target, and it is proposed that this target has to be the immune system itself, in the sense of a treatment that enhances the ability of the immune system to ward off viral infections independent of the viral genome. A mechanism of such a therapy has been described, where the target is the beta-receptor on cells of the immune system, and as a consequence, the cAMP-PKA pathway that inactivates and disables cells of the immune system. The mechanism is therefore an immunoactivation via inhibition of an inhibitory stress-related cellular pathway activated by beta-receptors. Various experiments have shown that this mechanism diminishes viral titers of different viruses and this has also been demonstrated in virus-infected patients treated with selected beta-blockers like propranolol. Beta-blockers used for such a therapy may need to have special properties like efficient penetration of the blood-brain barrier, they should be highly lipophilic and non-selective for beta-receptors.

A Cost-Benefit Approach to Sample Size Determination for Clinical Trials with Binary Responses

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The determination of sample size is an important issue in designing any trial. Particularly it is of key importance in medical studies. Many authors have looked at the problem and many papers have been written to discuss the problem form both the frequentist and Bayesian standpoints. In the frequentist approach sample sizes are usually determined either from power and size calculations or from formulae based on confidence interval widths. In the fully Bayesian (or decision theoretic) approach, as in this work, the sample sizes are determined by maximizing expected net benefit.

In this work we discuss a decision theoretic or fully Bayesian approach to the sample size question in clinical trials with binary responses. Data are assumed to come from two binomial distributions for which p_1 and p_2 are the probabilities of favourable outcomes for each individual in group i, (i=1, 2). To describe our prior knowledge about (p_1, p_2), we assume that it has a joint density. With a binomial likelihood it is mathematically convenient, and often reasonably realistic, to make the assumption that (p_1, p_2) has a Dirichlet distribution. The parameter of interest is p= p_1-p_2. The optimal size of the trial is obtained by maximizing the expected net benefit function which is the expected benefit of conducting the trial minus the cost of it.

Metals as Endocrine Disruptors in Women's Reproduction: Assessment of Effect and Mechanism of Action in Different Steroidogenic Cells

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Background: The polluted environment contains a mixture of reproductive toxicants that includes metals and metalloids. The emerging evidence exists that cadmium, lead, arsenic, mercury and the others can act as endocrine disrupting chemicals in mammals. They can alter ovarian and/or placental steroidogenesis and thus affect ovarian cyclicity, the maintenance of pregnancy, and embryo/foetal development. A better understanding of the endocrine disrupting potential of metal exposure bears great clinical relevance, as metals constitute an important part of our ecosystem and lifestyle, the production and use of which is unlikely to be discontinued in the foreseeable future.
Methods: We conducted complementary research on cadmium-related steroid disruption using different experimental paradigms. Human placentas were used for *ex vivo* (epidemiological) and *in vitro* studies of cadmium effect(s) on placental progesterone production. In experiments on laboratory rats *in vivo* and *in vitro* and in the stable porcine granulosa cell line JC-410, steroidogenesis was assessed in placental and ovarian steroidogenic cells.
Results: In either human or rodent placenta and in ovary, increased cadmium concentrations in steroidogenic tissue were accompanied by decreased progesterone production. Direct cadmium effects on specific components of the steroidogenic pathway were found. This includes two sites of action: the low-density lipoprotein-cholesterol receptor and P450 side chain cleavage enzyme. In cultured porcine granulosa cells, cadmium stimulated ovarian progesterone synthesis through a mechanisms involving activation of P450 side chain cleavage gene expression.
Conclusions: 1) Cadmium has the potential to disrupt steroidogenesis in human placenta. 2) Cadmium may display paradoxical dual effects in the ovary; depending on the exposure level, it may either inhibit or enhance/mimic the biosynthesis of progesterone and oestrogen, and act as xenoestrogen (metalloestrogen). 3) Sites of cadmium direct effects on specific components of the steroid biosynthetic pathway are multifaceted and it is possible that cadmium's effects are "tissue-specific" in different steroidogenic cells.

Cardiological medicines: allowed and prohibited pharmacological helping for athletes

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Background: Cardiological medicines are recommended athletes with medical indications by doctors working in a Center of Sport Medicine in Warsaw, Poland. There is almost two hundred consultations a year. The Therapeutic Use Exemption (TUE) allows athletes to use substances and methods banned by World Anti-Dopnig Agency (WADA). Diuretics are prohibited for sportsmen at all time (in and out of competitions). B-blockers are not allowed only in a particular sports (for example: ski jumps, snowboard or aerial and car sports) .On the other hand I-blockers or cardioprotecitve substances like a trimetazidine are not yet present on the WADA list of prohibited substances.
Methods: Department of Anti-Doping Research of Institute of Sport in Warsaw, Poland (WADA accreditation since 2004) had been analyzed 6210 urine samples of Polish athletes since 2005 to 2007. This material has been tested retrospectively in direction of finding diuretics, b-blockers and their metabolites, I-blockers and cardioprotective substance - trimetazidine.
Results: -diuretics were found in 7 samples
-b-blocker – only one case
-I-blocker –buflomedil was present in 6 athlete's samples (all cycling riders in competition)
-trimetazidine was found in 34 samples from various discipline of sport:
Strength discipline: trimetazydine was often found (n=27)
- 13 cases - cycling riders
- 7 cases – athletics
- 4 cases – triathlon
- 2 cases -canoeing
- 2 cases – swimming

Then in the forces (n=7):
- 3 cases - weigh-lifting
- 3 cases - football
- 1 case - judo

Conclusions:
1. The use of diuretics and b-blockers by athletes is the occasional phenomenon in Poland compared to the use of other banned substances such as anabolic steroids.
2. Trimetazidine and buflomedil are used as allowed pharmacological helping for athletes in Poland
3. There is no objective evidence of clinical trials of trimetazidine and buflomedil use in improving the physical capacity at the athletes.
4. The use of trimetazidine and buflomedil in enhancing the capacity of physical athletes, wakes up ethical doubts but is not prohibited.

Psychotomimetic Action of Ketamine and MK-801: BehavioraL, Network and Cellular Features

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Cognitive dysfunction, hallucination and delusion are typical disorders that are diagnosed in schizophrenic patients and that can be induced in humans following the administration of a single non-anesthetic dose of ketamine, a non-competitive NMDAr antagonist. What is the impact of a similar single injection of ketamine in the EEG of rodents? And what is the direct effect of ketamine in cortical networks?

In awaked adult rats, a single subcutaneous injection of ketamine (<5 mg/kg) induces persistent aberrant gamma frequency (30-80Hz) oscillations (increased power and intrinsic frequency) in the frontoparietal cortex and a concomitant ataxic behaviour. EEG recordings performed in deeply anesthetized rats demonstrated that these pathophysiological gamma oscillations are not caused by abnormal motor activity. Neither are they dependent on conscious sensorimotor processing. Local application of ketamine produces a cortical focus of persistent aberrant gamma oscillations, which progressively spread to adjacent networks. Furthermore, ketamine significantly increases the synchrony of basal gamma oscillations between two highly and not between two weakly interconnected structures. Juxtacellular recordings combined with EEG recordings have revealed that aberrant gamma oscillations are associated with a significant increase in the firing rate in the majority of glutamatergic corticofugal and thalamocortical neurons.

Our findings suggest that ketamine-induced persistent gamma hypersynchrony is an aberrant network noise that might set out of control the spatiotemporal patterning of inputs in cortical-related networks, which would impair top-down processing. A persistent decrease or annihilation of the signal/noise ratio of the cognition-related transient gamma synchrony might cause cognitive dysfunction, acute psychosis and exacerbate schizophrenia symptoms. Therefore, this persistent aberrant gamma noise may be a potential neurophysiological marker of psychoses.

INSERM and ULP support.

Targeting the Tumor Microenvironment as a Modality to Combat Cancer – Effect of Halofuginone

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Background: Most solid tumors comprise neoplastic and non-neoplastic cells plus extracellular matrix (ECM) components. This cellular microenvironment favors tumor development, and the ECM−stromal cell interactions contribute to the neoplastic phenotype. The tumor–dependent conversion of fibroblasts to myofibroblasts, mediated by transforming growth factor-beta (TGFβ), results in overproduction of ECM proteins. Thus, the fibroblast-to-myofibroblast transition has emerged as a viable target for pharmacological intervention. Halofuginone, an analog of the plant alkaloid febrifugine, inhibits Smad3 phosphorylation downstream of the TGFβ signaling. In pre-clinical and clinical studies halofuginone prevented new, and stimulated resolution of pre-existing fibrosis in which excess of ECM hallmarks the disease.
Methods: Xenografts were established by implanting various human tumor cells – subcutaneously or orthotopically – into nude mice. Tumor size was evaluated directly or by NMR micro-imaging. Gene expression and protein synthesis were evaluated by *in situ* hybridization and immunohistochemistry, respectively.
Results: Halofuginone, however administered and irrespective of cancer type, inhibited smad3 phosphorylation, resulting in inhibition of the fibroblast-to-myofibroblast transition, reduction in ECM production and reduction in angiogenesis resulted in inhibition of tumor growth. In prostate cancer xenografts representing various phenotypes of the disease, halofuginone inhibited tumor progression in correlation with reduction of plasma prostate-specific antigen. Halofuginone is ideal for combination therapy because of its unique mode of action and the dissimilarity of its targets from those of the conventional chemotherapies. In various xenografts, halofuginone synergizes with chemotherapy and reduces the need for high doses of toxic compounds, and thereby can reduce cancer patients' treatment burden without impairing treatment efficacy. Halofuginone is now undergoing clinical trials.
Conclusions: The TGFβ signaling cascade is shared by myofibroblasts in wound healing, fibrosis and cancer. In fibrosis where the myofibroblasts are the major participant halofuginone can be used alone; in cancer it should be considered in combination with other therapies that affect tumors via different modalities.

Anabolic Androgenic Steroids (AAS) Elicit Aggression by Selectively Decreasing Neurosteroid Biosynthesis in Corticolimbic Glutamatergic Neurons

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Background: AAS abuse is a serious problem among adolescents, elderly subjects, and military personnel. AAS increase sex drive and mental acuity but have remarkable physical and behavioral side effects, including irritability, aggression, and depression. The molecular mechanisms and neuronal circuitry underlying these symptoms are largely unknown. Protracted testosterone propionate (TP) administration in mice induces aggression accompanied by a decrease in brain allopregnanolone (Allo) content. Allo is a neurosteroid that positively and allosterically modulates $GABA_A$ receptor function. In the brain, Allo is synthesized from progesterone by the sequential action of 5α-reductase type I (5α-RI) and 3α-hydroxysteroid dehydrogenase (3α-HSD). 5α-RI and 3α-HSD colocalize in cortical, hippocampal, and olfactory bulb glutamatergic neurons and in output neurons of the basolateral amygdala (BLA), thalamus, and striatum.
Methods: To study the alterations of selective neuronal circuitry in AAS abuse, we established a mouse model of AAS-induced aggression. Using GC-MS, we studied whether TP, in doses that induce aggression, selectively downregulates Allo in a structure-specific manner. Using immunohistochemistry, quantitative nested RT-PCR, and Western blot, we studied whether TP decreases corticolimbic Allo levels by downregulation of 5α-RI mRNA and protein expression. We used local microinfusions of the selective brain steroidogenic stimulant (SBSS), S-norfluoxetine (S-NFLX), to upregulate Allo and decrease aggression.
Results: Treatment of mice with TP reduces 5α-RI expression in selected glutamatergic pyramidal neurons of the cortex, hippocampal CA3, and BLA and in granular cells of the dentate gyrus, which results in a decrease of Allo content. In contrast, 5α-RI mRNA expression fails to change in the striatum medium spiny neurons and reticular thalamic nucleus neurons, which are GABAergic. A bilateral microinfusion of S-NFLX in the BLA decreased TP-induced aggression by upregulation of Allo levels.
Conclusions: TP-induced aggression is associated with an impairment of Allo biosynthesis in specific corticolimbic glutamatergic neurons. These results may help in the design of therapeutics to limit the adverse effects of AAS abuse.

IL-12 receptor expression and function on human lung adenocarcinoma: identification of a new potential therapeutic target

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Background: Non small cell lung cancer (NSCLC) is a leading cause of cancer death. We have shown that mice lacking expression of the β2 chain of the interleukin-12 receptor (IL-12Rβ2 KO mice) develop spontaneously lung adenocarcinomas or bronchioalveolar carcinomas. Here we have investigated i) IL-12Rβ2 expression in human primary lung adenocarcinomas and normal bronchial epithelial cells (NBEC), and ii) the direct activity of IL-12 on NSCLC cells and the mechanisms involved.
Methods: Lung adenocarcinoma tissues obtained at diagnosis from seventy untreated patients were studied for IL-12Rβ2 expression by immunohistochemistry. Stage I lung adenocarcinomas showed significantly ($P=0.012$) higher frequency of IL-12Rβ2$^+$ samples than stage II/III tumors.
Calu6 NSCLC cells were next transfected with IL-12Rβ2 containing plasmid (Calu6/β2), while NBEC cells were expanded from lung specimens of non neoplastic origin. *In vitro* IL-12 activity on Calu6/β2 or NBEC was investigated by flow cytometry, ELISA and chorioallantoic membrane assay. Severe combined immune deficienty (SCID)/ non obese diabetic (NOD) mice were inoculated with Calu6/β2 cells subcutaneously or orthotopically and treated with human recombinant (hr) IL-12. Explanted tumors were studied by polymerase chain reaction (PCR) array and immunohistochemistry.
Results: IL-12 treatment of Calu6/β2 cells inhibited IL-6 production and angiogenesis *in vitro*. Tumors formed by Calu6/β2 cells in SCID/NOD mice were significantly smaller following hrIL-12 *vs* PBS treatment due to inhibition of angiogenesis. NBEC expressed functional IL-12R and IL-12 damped the release of cytokines involved in tumor progression.
Conclusions: 1) IL-12 inhibits directly the growth of human lung adenocarcinoma and targets the adjacent NBEC, 2) IL-12Rβ2 on primary lung adenocarcinoma may represent a new therapeutic target, and 3) clinical trials investigating hrIL-12 activity in lung adenocarcinoma patients appear feasible.

In Search of the Magic Bullets: Discovery of RN5 and its Structural Modifications for Targeting Selectively α_1 Adrenoceptors

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Background: Alfa1 adrenoceptors (α_1-ARs), further subdivided into α_{1A}, α_{1B}, and α_{1D} subtypes, are therapeutically relevant because represent pharmacological targets for a number of drugs currently used in the treatment of two widely occurring diseases, hypertension and benign prostatic hyperplasia (BPH).
Aims: 1) To develop new ligands highly selective towards α_1-ARs with respect to 5-HT_{1A} serotonin and dopaminergic D_1 and D_2 receptors. 2) To develop selective ligands with respect to the different □$_1$-AR subtypes to finally clarify the pharmacological profile of each receptor type and subtype.
Methods: Design and synthesis of pharmaceutically active molecules, Molecular modeling studies and pharmacological assays. Synthesis of radiolabelled 3-[2-[4-(2-[^{11}C]methoxyphenyl)piperazin-1-yl]ethyl]pyrimido[5,4-b]indole-2,4-dione, [^{11}C]RN5, for positron emission tomography (PET) studies on α_1-ARs.
Results: In this general overview of our twenty years research on adrenergic receptors, example of the most relevant medicinal chemistry results achieved are given. In the two last decades, we have been involved in the development of new selective α_1-AR ligands characterized by a planar tricyclic or bicyclic system coupled to a pharmacophoric phenylpiperazine (PP) moiety. Among more than one hundred synthesized ligands, 3-[2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl]pyrimido[5,4-b]indole-2,4-dione, RN5 is one of the most representative example of such ligands. Nowdays, this compound is sold and commonly used as standard reference ligand for α_1-ARs. A number of RN5 analogues were subsequently synthesized and even more selective α_1-AR and high affinity ligands were identified with respect to other related receptors. Moreover, some interesting subtype selective ligands were designed and synthesized. Chemistry, structure-activity relationships, molecular modeling studies, and pharmacological results of these α_1-AR ligands will be discussed.**Conclusions:** 1) RN5 is one of the most representative ligands discovered. 2) [^{11}C]RN5 radioligand was synthesized and *in vivo* PET biodistribution of α_1-ARs was studied. 3) A number of RN5 analogues with improved profile were synthesized. 4) Moreover, some subtype selective ligands were identified.

Emergence of Colistin Resistance during Therapy of Infections by Multi-drug Resistant Gram Negative Pathogens in the Critically Ill

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Background: Selective pressure due to colistin (Col) may result in emergence of Col resistance among multi-drug resistant gram negative bacteria (MDR-GNB), jeopardizing treatment choices in the ICU. The aim of this study was to present the emergence of Col resistant (ColR) MDR-GNB isolated from ICU patients in association with exposure to Col.
Methods: The study was performed in a 12-bed University General ICU from November 2003 to December 2006. Empirical antimicrobial treatment was guided by weekly active surveillance of patients' floras. All specimens were cultured in MacConkey agar plates containing antibiotics in order to focus on resistant pathogen detection. ColR was defined by Etest according to BSAC breakpoints (>4ig/ml). Epidemiological typing was performed by Rep-PCR in the fist sensitive and the first ColR isolate from each patient.
Results: 152 patients were included in the study. Patients were colonized by *K.pneumoniae* (54%), *P.aeruginosa* (57%), *A.baummanni* (75%), *Enterobacter spp* (17%), *E.coli* (28%) and *S.maltophilia* (30%) while ColR strains were 37%, 4.5%, 2.5%, 4%, 7.3% and 44% respectively. Among patients colonized with at least one ColR strain, 95% had been exposed to Col while among patients who had been exposed to Col, 66% developed at least one ColR strain. Epidemiological typing demonstrated 20 distinct clones of ColR *K.pneumoniae* isolated from 30 patients. The median duration of exposure to Col, for patients that were colonized or infected by distinct ColR *K.pneumoniae* clone during their hospitalization was 18 days. Epidemiological typing showed that there was horizontal transmission of the resistant clone among co-hospitalized patients. 18.5% of patients were also colonized with an intrinsically ColR enterobacteriacae; among them 93% had been exposed to Col ($p<0.01$, OR:11.66).
Conclusions: 1) Unnecessary and/or prolonged (>2weeks) Col administration is associated with colonization of ColR MDR-GNB 2) Both endogenous acquisition and horizontal transmission are responsible for the spread of Col resistance in the ICU environment under selective pressure.

Silencing Cell-Cell Communication: The New Bullets in Anti-Infectives

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Background: At least two lines of communication occur in the host: bacterial cell-cell communication (quorum sensing) and cross-kingdom communication, i.e., host hormones functioning as molecular signal mimics. Both forms of chemical communication can affect phenotypic expression of virulence factors and antimicrobial susceptibility. Currently, focus is on design of quorum sensing autoinducer analogs. However, cross-kingdom communication presents a particularly intriguing line of communication, with evolutionary origins of some host hormones hypothesized as microbial. To determine their putative role as inter-kingdom communication molecules, the effect of insulin and dehydroepiandrosterone (DHEA) on phenotypic expression was assessed.
Methods: *Staphylococcus aureus* 29523 grown with DHEA (0.1, 0.5, 1.0, 5.0 μM) was tested for susceptibility to Triton X-100, lysozyme, β-defensin and vancomycin. r-Insulin's (Humulin) role as a microbial insulin mimic was tested in *Escherichia coli*' K12 by measuring the effect of insulin (2-400 μU/ml) on *E. coli* adherence, biofilm production and chemotaxis, using standard methodology.
Results: *S. aureus* exposure to DHEA resulted in increased carotinoid levels which correlated with increased resistance to Triton-X100, lysozyme, β-defensin and vancomycin. Exposure of *E. coli* to r-insulin affects *E. coli* behavior. Insulin alone is a chemo-repellent. However, with glucose insulin enhances glucose chemoattraction, adherence (glass, plastic and latex) and biofilm formation as compared to sugar alone.
Conclusion: 1) DHEA enhances carotenoid synthesis and resistance to cell-wall active agents. 2) r-Insulin in the absence of glucose disperses populations in nutritionally impoverished environments; however, with glucose present insulin enhances population density through biofilm formation. 3) It is possible that in some cases inter-kingdom signaling molecules may provide an alternative signaling pathway that will need consideration along with more traditional quorum autoinducers if analogs of this class of compounds will have broad utility as anti-infectives.

Treating fungal infections with CYP53A15 inhibitors

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Our in vitro and in vivo studies suggest that targeting CYP53A15 could help treat fungal infections. In vitro, four phenolic compounds (isoeugenol, eugenol, vanillin and thymol) that play a role in plant resistance to fungal infection inhibited CYP53A15. Inhibition of CYP53A15 leads to increased intracellular levels of benzoic acid, which impedes fungal growth. In vivo, three of these compounds inhibited Cochliobolus lunatus growth. Next steps include solving the X-ray crystal structure of the CYP53A15 active site.

Ref. CYP53A15 of Cochliobolus lunatus - a target for natural antifungal compounds, J. Med. Chem.; published online May 28, 2008; doi:10.1021/jm800030e

Status and antibiotic sensitivity profile of *Salmonella enterica during* 2001-2007 at Tribhuvan University Teaching Hospital - a tertiary health care centre of Nepal

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Background: Enteric fever is a common problem in Nepal. In view to determine the trends of *Salmonellae* causing bacteremia/septicemia and to determine the antimicrobial susceptibility profile of these organisms, a retrospective study was carried out during 2001 to 2007 at Tribhuvan University Teaching Hospital, a tertiary care health centre inNepal.
Methods: During 2001-2007, 41408 blood culture samples from the patients clinically suspected of enteric fever were received at Department of Microbiology,Tribhuvan University Teaching Hospital, Katmandu, Nepal. These samples were cultured and isolates were identified then subjected for antibiotic sensitivity testing as described by American Society for Microbiology (ASM).
Results: Approximately 13% blood culture samples showed growth positive. Of which about 10% were *Salmonella enterica serotype Typhi and Paratyphi-A*. Three percentage of isolates included *Staphylococcus aureus, E.coli, Klebsiella pneumoniae, Pseudomonas spp, Viridans Streptococci, Acinetobacter calcoaceticus, Citrobacter spp, Enterobacter spp and Streptococcus faecalis*. Among 4013 *Salmonella* isolates, 6.5% were resistant to at least two groups of antibiotics. Almost equal percentage of both *salmonella enterica serotype Typhi and Paratyphi-A* isolates were found to be multi drug resistant (MDR). Many of the multi drug resistance *Salmonella*e were resistant to ampicillin, ciprofloxacin, co-trimoxazole and chloramphenicol.
Conclusion: There is increasing trends of Multi drug resistant *Salmonellae* over the years, therefore It is felt that the cause of resistance should be investigated.

***N*-Oleoyl-Dopamine: A Potential Novel Deliverer of Dopamine to the Brain**

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Background: *N*-oleoyl-dopamine (OLDA) is the most biologically active *N*-acylated dopamine, a member of a new class of lipid compounds, termed dopamides. The compounds are a condensation product of dopamine (DA) and a polyunsaturated free fatty acid chain. We hypothesized that a lipophilic OLDA could serve as a carrier of DA into the biomembranes of neural cells, the target site of cellular signaling. DA has a well defined role of a major neurotransmitter in both the brain and the carotid body; the latter is an organ of neural crest origin that generates the hypoxic ventilatory response. Aims: 1) To study the uptake of radiolabeled *N*-OLDA by the brain, a barrier-equipped, and the carotid body, a non-barrier- equipped organ, and to compare it with that of radiolabeled DA alone. 2) To study the effect of *N*-OLDA on the hypoxic respiratory response. 3) To ascertain dopaminergic mediation in *N*-OLDA respiratory effects.
Methods: The study included a total of 28 anesthetized Wistar rats, weight: 180-340 g. One group, 22 rats, received intracarotid injections of 0.3 ml [^{3}H]*N*-OLDA dissolved in DMSO, 1.33 µCi/ml and 1 ml [^{3}H]DA dissolved in NaCl, 10 µCi/ml. The ^{3}H radioactivity was measured by a scintillation counter. The rats of the other group were paralyzed, ventilated, and respiratory neural output was recorded from the integrated phrenic nerve activity. The ventilatory response to 14 and 11% O_2 in N_2 was taken at baseline, after OLDA-20 mg/kg, ip, and then after a DA D2 receptor antagonism by Haloperidol-300 µg/kg, iv.
Results: OLDA was taken up by both neural tissues studied. The regional brain uptake of [^{3}H]*N*-OLDA was ~6% and the carotid body uptake was ~30% of the entire radioactivity injected, both being 3-4-fold greater than those of [^{3}H]DA alone in respective tissues (P<0.01). The peak stimulatory hypoxic respiratory response was diminished by OLDA by ~20 and 45% during the responses to 14 and 11% hypoxia, respectively. The inhibitory effect of *N*-OLDA was abolished by pretreatment with Haloperidol.
Conclusions: 1) OLDA has an inhibitory, DA-like effect on the hypoxic chemoreflex. 2) OLDA is incorporated into DA-mediated signal transduction in neural tissues. 2) OLDA may serve as a carrier of DA into neural tissue. 3) OLDA's potential to stabilize DA molecule in biomembranes may give rise to its sustained action, as opposed to the fleeting effects of DA proper.

The Use of Regulatory RNA Molecules as a Novel Treatment Strategy for Cardiac Diseases - RNA as a Magic Bullet ?

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The current status and challenges of RNA interference (RNAi) and microRNA modulation strategies for the treatment of myocardial disorders are discussed and related to the classical gene therapeutic approaches of the past decade. We summarize the key issues of current vector technologies which determine if they may be suitable for clinical translation of experimental RNAi or microRNA therapeutic protocols. We then present and discuss examples dealing with the potential of cardiac RNAi therapy. First, an approach to block a key early step in the pathogenesis of a virus-induced cardiomyopathy by RNAi targeting of a cellular receptor for cardiopathogenic viruses. Second, an approach to improve cardiac function by RNAi targeting of late pathway of heart failure pathogenesis common to myocardial disorders of multiple etiologies. This strategy is directed at myocardial Ca2+ homeostasis which is disturbed in heart failure due to coronary heart disease, heart valve dysfunction, cardiac inflammation, or genetic defects. Whereas the first type of strategies (directed at early pathogenesis) need to be tailor-made for each different type of pathomechanism, the second type (targeting late common pathways) has a much broader range of application. This advantage of the second type of approaches is of key importance since enormous efforts need to be undertaken before any regulatory RNA therapy enters the stage of possible clinical translation. If then the number of patients eligible for this protocol is large, the actual transformation of the experimental therapy into a new therapeutic option of clinical importance is far more likely to occur.

Modern quantum chemical descriptors for QSAR/QSPR

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Background: The current availability of cheap computing power enables solving the Schrödinger equation for congeneric drug-sized molecules. Modern *ab initio* methods yield electronic properties of geometry-optimized molecules. A new theory called Quantum Chemical Topology (QCT) (based on "Atoms in Molecules") provides descriptors for QSAR/QSPR. These modern and more realistic descriptors capture electronic effects and replace the Hammett constants.
Methods: QCT locates Bond Critical Points (BCPs) in 3D space. Properties such as the electron density or its Laplacian describe the bonds. A molecule is compactly represented in an abstract space of BCP properties[1]. Partial Least Squares and various machine learning techniques can map the QCT descriptors to a wide variety of activities of medicinal, ecological (toxicological) interest. Cross-validation and randomisation testing protect against chance correlation. True external validation features in our recent studies. Our method can localise a part in the molecule where the chemical change associated with the observed activity actually happens.
Results: In the case of the antitumour activity of phenylbutenones we confirmed[2] the hypothesis that these compounds act via a Michael addition. In the context of mutagenic activity[3] we determined a preferred mechanistic pathway for the initial hydroxylation of dimethyl heteroaromatic triazenes, a hitherto ambiguous issue. We studied[4] seven datasets: (1) pK_a of substituted imidazolines and (2) imidazoles, (3) the ability of indole derivatives to displace [^{3}H] flunitrazepam from binding to bovine cortical membranes, (4) the influenza inhibition constants for benzimidazoles, (5) the interaction constants for amides and the enzyme liver alcohol dehydrogenase, (6) the natriuretic activity of sulfonamide carbonic anhydrase inhibitors and (7) the toxicity of benzyl alcohols. Hepatoxicity[5] and nitroaromatic toxicity[6] were also investigated.
Conclusions: Electronic effects can be captured by a novel class of QCT descriptors that come directly from modern ab initio wavefunctions. Active sites in molecules are highlighted and robust predictions made for diverse activities.

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Pharmacokinetic and pharmacodynamic interactions between mepivacaine and antihypertensive drugs

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Background: Aims: To analyse 1) the pharmacokinetic interaction between mepivacaine and propranolol and 2) pharmacodynamic interactions between mepivacaine and some antihypertensives drugs.
Methods: 1) A randomised, double blind, cross-over pharmacokinetic study included 10 male normotensives. Each subject received 51 mg mepivacaine for dental local anaesthesia (LA) two hours after he ingested 30 mg of propranolol or placebo. Mepivacaine concentration in venous serum was measured after 5, 15, 30, 45, 60 minutes from injection by gas chromatography. 2) A pharmacodynamic study included 62 male patients, divided in 3 groups: normotensives, nontreated hypertensives, treated hypertensives (with enalapril, propranolol, or verapamil). They were tested for dental sensitivity, blood pressure (BP) and cardiac rate before and after a local anaesthesia with mepivacaine 3% for a maxillary tooth.
Results: 1) Peak serum concentrations of mepivacaine, Cmax, (1.214 +/- 0.746 μg · mL-1) were significantly increased by propranolol (2.249 +/- 1.559 μg · mL-1, $p < 0.05$). 2) Pain intensity determined by mepivacaine injection (measured with visual analogue scale) varied in this order: untreated hypertensives < treated hypertensives < normotensives. Latency of pulpal LA was under 5 minutes, and latency of soft tissue LA was under 2 minutes for all groups. Duration of LA varied in the following order: normotensives < enalapril hypertensives < untreated hypertensives < propranolol hypertensives < verapamil hypertensives. In verapamil and also propranolol treated hypertensives groups, duration of LA was significantly longer then for normotensives and untreated hypertensives. BP dropped significantly 90 minutes after anaesthesia in normotensive group and treated hypertensives, while for nontreated hypertensives it slightly changed. Cardiac rate did not varied significantly in all groups.
Conclusions: There are pharmacokinetic interactions between mepivacaine and propranolol that could explain the effect of propranolol of increasing mepivacaine toxicity. There are also pharmacodynamic interactions between mepivacaine and antihypertensives, manifested especially for LA parameters, verapamil and propranolol increasing the duration of local anaesthesia.

The Role of the Genotype in the MAGIC BULLET Effect of Psychotropic Drugs

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Background: Recent progress in neurogenomics has opened up new lines of research in the crucial pharmacologic problem - the mechanisms underlying the difference in individual sensitivity to drugs. The present experiments tested the hypothesis that critical mechanism in the response to psychotropic drugs involves genetically defined serotonin 5-HT receptor expression. Aims: 1) To elucidate the effect of differences in the 5-HT_{1A} receptor gene expression and the density of 5-HT_{1A} receptors on the sensitivity to 5-HT_{1A} agonist 8-OH-DPAT. 2) To compare the effect of chronic imipramine treatment on 5-HT_{2A} receptor mRNA level in genetically predisposed to catalepsy and noncataleptic rats.
Methods: The study included 85 Norway rats selectively bred for high level and for the lack of fear-induced aggression, 27 rats selectively bred for predisposition to catalepsy (GC) and 28 Wistar rats. Specific binding of [3H]8-OH-DPAT and 5-HT_{1A} and 5-HT_{2A} receptor mRNA levels were estimated. As functial correlates for 5-HT_{1A} receptors, 8-OH-DPAT-induced (0.5 mg/kg i.p.) hypothermia and lower lip retraction (LLR) were used. Imipramine (15 mg/kg per day) was given to Wistar and GC rats in drinking water for 27 days.
Results: 8-OH-DPAT produced a distinct hypothermic reaction in nonaggressive Norway rats and did not affect the body temperature in aggressive rats. Similarly, LLR was expressed much more in nonaggressive than in aggressive animals. Considerable differences between the highly aggressive and the nonaggressive rats were shown in the 5-HT_{1A} receptor gene expression and 5-HT_{1A} receptor density in the brain regions. A significant decrease in Bmax of specific binding of [3H]8-OH-DPAT in the frontal cortex, hypothalamus, and amygdala and a reduction in 5-HT_{1A} receptor mRNA level in the midbrain of aggressive rats compared to nonaggressive were found.
In genetically predisposed to catalepsy GC rats, 5-HT_{2A} receptor mRNA level in the frontal cortex was lower than in control Wistar rats. Chronic imipramine treatment attenuated catalepsy and produced two-fold increase in 5-HT_{2A} receptor mRNA level in GC rats without any effect on 5-HT_{2A} receptor expression in Wistar.
Conclusions: 1) MAGIC BULLET effect depends on genetically defined state of the target. 2) The 5-HT receptor gene expression is essential for response to psychotropic drugs.

Sperm immobilization factor: potential candidate for fertility control and antibacterial targeting bacterial motility

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Unplanned pregnancies present a great risk to the reproductive health of women. Therefore female-controlled vaginal products directed towards contraception are needed urgently. In the present study efforts have been made to evaluate the contraceptive potential of sperm immobilization factor (SIF) isolated from *Staphylocccus aureus* isolated from an infertile woman. The results showed that sperm motility was completely inhibited by SIF along with complete loss of viability. The effect on the sperm motility was found to be dose and time dependant. The minimum effective concentration of SIF required for complete immobilization of spermatozoa (40 x 10^6) in vitro within 20 s was found to be 150μg. Intravaginal administration of SIF (50μg) before mating during proestrous-estrous transition phase caused complete blockage of conception in mouse model. Sub-acute toxicity studies in mice indicated that repetitive intravaginal application of SIF at a dose of 100μg for 14 consecutive days induced no abnormality either in length of the estrous cycle or in the morphology of the vaginal tissue. Furthermore, no adverse effect was observed on subsequent reproductive performance, neonate survival and development of pups. In addition, SIF was also found to induce immobilization in various motile bacteria in vitro viz. *Escherichia coli, Pseudomonas aeruginosa* and *Proteus mirabilis* which are known uropathogens. 6-8 h old cultures of these bacteria when incubated along with SIF caused 100% immobilization. As motility and adherence represent an integral aspect of bacterial pathogenesis, therefore, targeting bacterial motility can be exploited as a potential antibacterial therapy. Though, antibiotics have been used ever since their discovery. However, the complications involving the emergence of multi-drug resistant strains and causing chronic toxicity pose a challenge for modern medicine. Because of public concern, now the focus has shifted towards the use of safer therapeutic agents. Newer biocompatible agents including the microorganisms and their products are being examined for their potential as antimicrobials. It is suggested that SIF could be developed as a potent vaginal contraceptive as well as antibacterial agent targeting bacterial motility for future use in humans.

Exploring Leukotriene B_4 (LTB_4) as a periodontal biomarker: A time to focus.

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Background: Leukotriene B_4 (LTB_4) is a membrane-derived lipid mediator formed from arachidonic acid. LTB_4 is among the most potent stimulants of polymorphonuclear leukocytes (PMNs) and, thus, participates in tissue injury by recruiting PMNs in a pathophysiologic scenario of periodontal and other systemic diseases. The aim of the present study was to assess the relationship between clinical parameters and concentrations of LTB_4 within gingival crevicular fluid (GCF) from inflamed gingiva and periodontitis sites before and after the treatment of periodontitis.
Methods: Sixty subjects were divided into three groups with 20 subjects in each group: healthy (group 1), gingivitis (group 2), and chronic periodontitis (group 3). Groups were based on periodontal disease index (PDI), clinical attachment loss (CAL), and radiographic evidence of bone loss. Group 4 consisted of the subjects in group 3 at 6 to 8 weeks after treatment, i.e., scaling and root planing (SRP). GCF samples collected from each patient were quantified for LTB_4 using an enzymatic immunometric assay. In addition, the correlation between in situ LTB_4 levels and clinical parameters was analyzed in each group.
Results: The highest mean LTB_4 concentration in GCF was observed in group 3 (185.2 pg/microl), and the lowest was observed in group 1 (39.6 pg/microl). Its level in group 3 decreased to 79.35 pg/microl after treatment (group 4). Further, GCF LTB_4 levels in all groups showed a statistically significant positive correlation with PDI and CAL ($P < 0.005$).
Conclusion: The substantial increase in GCF LTB_4 concentrations with the severity of periodontal disease and a concomitant decrease in its level following SRP in subjects with periodontitis suggest a possible role for LTB_4 as a biomarker in the progression of periodontal disease and aid in our understanding of the pathogenesis and the appropriate treatment of periodontal diseases by utilization of LTB_4 inhibitors and antagonists to LTB_4 receptor as "magic bullet" that would bypass the need for professional and self-administered oral prophylaxis protocols.

Cell-specific delivery of kinase inhibitors using lysozyme: A novel approach to treat renal fibrosis

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Background: Activation of renal tubular cells, mediated by several kinase pathways, is the key process in renal fibrosis. However, kinase enzymes regulate many normal physiological processes elsewhere. Therefore we developed a novel strategy to deliver kinase inhibitors selectively to renal tubular cells using lysozyme (LZM) as a drug carrier protein. This will increase therapeutic drug levels in target cells and reduce side-effects. Aim: 1) To deliver different kinase inhibitors, that is, p38 MAPK inhibitor SB202190, TGF-beta kinase inihibitor (TKI) and RhoA kinase inhibitor Y27632 to tubular cells using LZM. 2) To evaluate the efficacy of drug-LZM conjugates in renal fibrosis models in rats.
Methods: SB202190, TKI and Y27632 were conjugated to LZM using ULS™ linker. Drug-LZM conjugates were characterized for drug to protein ratio and stability in many matrixes using HPLC methods. The renal uptake of the conjugates was studied upto 72h after a single i.v. injection in rats using HPLC methods. The efficacy of the conjugates was examined in either unilateral ureteral obstruction model (UUO) or ischemia/reperfusion injury model (I/R) in rats. The antifibrotic effects were determined using RT-PCR and immunohistochemical techniques.
Results: All drug-LZM conjugates contained 1:1 drug to protein ratio. In vitro, the conjugates released drugs in kidney homogenates while remained stable in serum and buffers. In vivo, the conjugates were rapidly accumulated in renal tubular cells and slowly released their drug intracellularly upto 72h after a single i.v. injection. Treatment with TKI-LZM and SB-LZM reduced renal fibrotic markers in rats with UUO and I/R injury, respectively. Intriguingly, blockade of RhoA kinase pathway with Y27632-LZM in kidneys substantially inhibited I/R-induced inflammatory and fibrotic processes. Moreover, Y27632-LZM attenuated the transdifferentiation of tubular cells, a crucial process after renal injury.
Conclusions: 1) We have successfully delivered several kinase inhibitors selectively to tubular cells in kidneys using our novel strategy. 2) Drug-LZM rapidly deposited in the cells and released the active drug intracellularly for prolonged time. 3) Targeted conjugates significantly inhibited renal inflammation and fibrosis in different animal models.

Valproic Acid (VA) May Be Effective in the Treatment of Headaches Associated with Reactivation of Cerebral Toxoplasmosis (CT)

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Background: Valproic acid (VA) is effective in the treatment and prophylaxis of migraine with and without aura, refractory migraine headache, drug-resistant migraine, tension-type headache, chronic daily headache, and convulsive disorders. We found that different types of headaches were precipitated by various diseases or medications probably because marked immune irregularities associated with these events caused reactivation of CT. The aim of this study was to focus on pathomechanisms that may be responsible for these clinical effects.
Methods: Literature data were selected to illustrate that latent CNS T. gondii infection/ inflammation intensity and/or host defense mechanisms may be affected by changes in production of NO, cytokines, tryptophan degradation by indoleamine 2,3-dioxygenase, limiting the availability of intracellular iron to T. gondii, production of reactive oxygen/nitrogen species, mechanisms mediated by an IFN-γ responsive gene family, and finally cause reactivation of CT. It must be added that cytosolic calcium T. gondii tachyzoite levels.play an important role in their invasion to the host cells and intracellular replication.
Results: VA was found to induce generation of IL-1α, IL-1β, IFN-γ, IL-6, ROS, NO, and monocyte attractant protein-1. These irregularities could markedly affect both the host and T. gondii defense mechanisms important for immune control of the parasite, because the in vitro study showed that VA inhibited replication of tachyzoites at median concentration of 4.1 μg/ml, similar to that of trimethoprim (5,3 μg/ml). In addition, trimethoprim exerted a synergistic effect with VA which may suggest that the mechanisms of actions of these two drugs were different. This suggestion may be supported by the finding that VA is also capable of inhibiting calcium transport through cellular ion channels. Because VA brain levels in the epileptic patients are about 20% of serum concentrations, and it is known that the subjects with schizophrenia and bipolar disorder who orally receive VA therapy achieve its blood concentrations of 50-100 μg/ml, the drug may be effective also in treatment of CT.
Conclusion: VA may be effective in the treatment of CT, various types of headaches and convulsive disorders because it affects both the immune state of the host and/or the parasite, and decreases calcium ion available for T. gondii tachyzoites, important for their motility.

Clinical Importance of Antimicrobial Resistance

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Background: The World is facing the problems of growing antimicrobial resistance in almost all categories of human pathogens (bacteria, fungi, viruses and parasites), difficult to treat infections and slow drug development. The problem has become more complicated due to increase in immunocompromised and critically ill patients. Infectious complications associated with multidrug resistant (MDR) organisms lead to high morbidity and mortality, and increased health care cost. This update aims to review our experience on the growing problems of multidrug resistant bacteria and their clinical implications.
Methods: This study is data analysis of last 5 years on antibiotic resistance in clinically important bacterial pathogens at our centre and review of literatures. Resistance was determined following standard screening, confirmatory phenotypic and molecular methods.
Results: Isolation of pathogenic bacteria from different anatomic sites in 2003 through 2008 ranged from 26% to 33%. The increasing trends of bacterial resistant to almost all classes of antibiotics were observed. The resistance rate to important bacterial pathogens were as follows: extended spectrum ß-lactamase and AmpC ß-lactamase producing *Enterobactariaceae* 78%, metallo ß-lactamase producing *Pseudomonas* and *Acinetobacter* species 31% and 21% respectively, methicillin resistant *Staphylococcus aureus* 51%, vancomycin resistant entercocci 11%. Percent change in resistance rate for different pathogens over the period ranged from 6 to 18%. Prior use of antibiotics, presence of invasive devices, prolonged hospitalization, admission in ICUs and ventilatory support were associated with antimicrobial resistance. Mortality rate was reduced by almost 50% and hospital stay by several days in patients who received appropriate antibiotic therapy. Mortality was significantly higher in patients already colonized with MDR bacteria prior to infection.
Conclusions: The trends of bacterial pathogens resistant to almost all classes of antibiotics are increasing with adverse clinical outcome. The key to address this escalating antimicrobial drug resistance problem lies in: 1) judicious use of available antibiotics with the principle of "hit fast and hit hard" with appropriate antibiotics, 2) avoidance of excessive and unnecessary use of antibiotics and 3) adoption of stringent infection control measures.

In vitro hypothesis, in vivo veritas. Success and failure of Imatinib incancer target therapy

PRICL S

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Background: Imatinib is currently registered for two major indications: (a) monotherapy in chronic myeloid leukemia (CML) and (b) monotherapy in c-Kit (CD117)-positive unresectable or metastatic gastrointestinal stromal tumors (GISTs). Imatinib mechanism of action is to bind to specific tyrosine kinases (Bcl-Abl in CML and c-Kit in GISTs), thereby blocking the corresponsing signaling for cell growth and proliferation of malignant cells in CML and GIST. Imatinib is an effective drug; nevertheless, resistance developes over time in many patients. Although kinase overexpression and gene amplification have been observed, the most common event in resitance is the occurrence of mutation(s) in the corresponding genes.
Methods: Clinical, biochemical and molecular modeling analyses of some important successful/unsuccessful cases observed during imatinib therapy of GIST patients are reported. Transient transfection experiments with plasmids carrying different patients KIT acquired point mutations were performed along with immunoprecipitation of total protein extracts, derived form imatinib treated and untreated cells. The molecular mechanics/Poisson Boltzmann Surface Area (MM/PBSA) computational techniques were applied to study the interactions of the wild-type and mutated receptors with Imatinib at molecular level.
Results: KIT phosphorylation was detected in cells transfected with vectors carrying the specific mutant genes. Imatinib treatment demonstrated that some mutations were insensitive to the drug at all applied concentrations, while others were inhibited by imatinib, although to different extent. Modelling of the mutated receptors revealed some motations substantially modify the protein binding pocket, thus hampering inhibitor binding, whilst others induce only relatively confined structural changes, still compatible with drug binding.
Conclusions: The results obtained from the clinical/biochemical analysis on mutated receptors testing the actual imatinib inhibitory efficiency coupled with molecular modelling highlighted the streght and weakness of this inhibitor towards c-kit mutated isoforms. Therefore these investigation ensemble could be of help in the design of new drugs and give important information to medical oncologist indicating the most suitable dose for escaping secondary resistance.

The Discovery of New Scaffold Antibacterial Agents

PRIMEAU J

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At AstraZeneca a key strategy, used to discover and develop antibacterial agents that will address the growing challenge of bacterial resistance, has focused on the design of new chemical scaffolds for new or under-exploited bacterial targets. The application of a diversity of lead generation techniques such as HTS and fragment based screening (using NMR and high concentration techniques), supported by strong structure based design capabilities has provided a range of novel compound starting points that have yielded novel lead scaffolds for these targets. This presentation will describe some of our efforts to date to transform these novel lead scaffolds into compounds that display excellent on-target potency, antimicrobial activity and in more recent cases, efficacy in animal models bacterial disease.

Novel biologic therapies for psoriasis

PROHIC A

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Psoriasis is a chronic, inflammatory skin disease that usually necessitates treatment over the course of patient's lifetime. Although topical medications usually suffice, about 25% patients with moderate-to-severe psoriasis will need additional systemic therapy, phototherapy or both. The long-term continuous use of traditional systemic therapies such as methotrexate, cyclosporine, oral retinoids and phototherapy (PUVA, which is psoralen plus ultraviolet light) is often limited due to potential organ toxicity, myelossuppression and carcinogenicity. The recognition of psoriasis as a T-cell mediated disease has led to the development of the new biologic agents that specifically target key steps of the immune pathways. This therapeutic approach is in contrast to current systemic therapies that act predominantly on hyperkeratinizaton and epidermal infiltrate, or broadly and non-specifically suppress the immune system. Biologics are pharmacologically active proteins extracted from animal tissue or synthesized through recombinant DNA techniques. They are designed to mimic the action of normal human proteins or to interact with circulating proteins or cellular receptors. There are three distinct classes of biologic agents: monoclonal antibodies, fusion proteins and recombinant cytokines.
Several biologics agents are now recognized for the treatment of moderate to severe psoriasis and/or psoriatic arthritis. These agents can be categorized into two broad classes based on their mechanism of action: the T cell inhibitors (alefacept and efalizumab) and TNF-α inhibitors (etanercept, infliximab and adalimumab). The aim of these new therapies is to improve the treatment of psoriasis, particularly moderate to severe psoriasis, with agents that are well tolerated and safe for long-term use. Potential limitations in the use of biologic agents include the high annual costs for treatment, lack of long-term follow up and selective nature of the patient populations thus far.

Application of Prodrug Strategies to Create Magic Bullets for the Treatment of CNS Maladies

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Background: Finding pharmacological interventions that would truly meet the definition of magic bullet in treating maladies affecting the central nervous system (CNS) has been challenging. Targets for interventions are generally not specific in the CNS and, in addition, protected from uncontrolled exposure of substances delivered into the systemic circulation by the blood-brain barrier (BBB). Although many drugs cross the BBB and exert therapeutic CNS-effects, their systemic exposure that impacts the rest of the body is unavoidable.
Methods: Strategies that, along with in vivo conversion of an inactive precursor (prodrug) to the active drug, rely on the manipulation of both the influx and efflux of substances across the BBB or, alternatively, site-specific activation of the prodrug may create magic bullets for the CNS. In this presentation, design and *in vitro*/*in vivo* evaluation of prodrugs that confine the action of neuropeptides (with focus on thyrotropin-releasing hormone, TRH) and estrogens are featured as respective examples for these strategies.
Results: For hydrophilic compounds such as peptides that practically do not cross the BBB, prodrug design has been the most versatile strategy to target them into the CNS. Simultaneously controlling activity, metabolism, transport and target-site retention via chemical manipulations is the key for creating magic bullets from neuropeptides such as TRH with promise to treat various neurological diseases (motorneuron diseases, various forms of dementia and brain trauma). For lipophilic compounds such as estrogens, profound endocrine responses by off-target peripheral tissue burden therapeutic intervensions for disorders of central origin that could be ameliorated by them (menopausal symptoms, impaired cognition, ischemic stroke, etc.). The success of CNS-selective estrogen therapy by a prodrug approach rests on the proper alteration of BBB-transport properties along with the facilitation of specific metabolic conversion(s) in the target tissue *versus* systemic bioactivation.
Conclusions: Various ways of creating magic bullets for the treatment of CNS maladies by the prodrug approach have been conceived. Although much remains to be learned about their merits, progress in the field has been steady, which clearly warrants continued exploration and development.

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Cellular Immune Toxicity of Alcohol and Cocaine: Medical Practice Based on the Evidence vs. Evidence-Based Medical Practice

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In 1906, Prof. Ehrlich shared the Nobel Prize in medicine for his pioneering research on the immune system. Largely based on his seminal work, we now realize that immune responses are complex biological processes modulated by intertwined epigenetic, genomic, molecular, biochemical, cellular and physiologic regulatory mechanisms. When acting in concert and appropriately, these processes converge toward efficient, rapid and complete removal of the invading pathogen. Immune events are delivered by several cell populations that work in conjunction with soluble factors, including antibody species that recognize and target individual pathogens. Prof. Ehrlich termed this remarkably specific process the "magic bullet". Our understanding of how immunity works has improved significantly over the past decades, and the "magic bullet" theory is today the prevalent model for treatment intervention in infectious diseases, cancer, oral pathologies, and even certain neurodegenerative pathologies (e.g., Alzheimer's disease). Illicit drugs of abuse, often co-abused with alcoholic beverages, interfere significantly with magic bullet-driven treatment interventions. Reports describe the many benefits of detoxification programs, but do not concur with respect to immune ameliorations.
The contemporary model of clinical intervention is grounded on the systematic evaluation of the best available evidence, and the judicious integration of appropriately revised clinical practice guidelines in the evidence-based treatment decision-making process. We present a systematic review of the available evidence with respect to the immunotoxicity of alcohol and joint alcohol/cocaine abuse, and of the immune benefits of detox programs, followed by level of evidence analysis, acceptable sampling analysis and meta-analysis. We demonstrate the practical usefulness of our findings in the context of the novel evidence-based model for XXI Century medical practice.

Discovery of the HCV NS3/4A Protease Inhibitor, Boceprevir (SCH503034). Key Steps in Structure-Based Optimization.

PRONGAY AJ

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The Hepatitis C Virus (HCV) infects about 200 million people worldwide. The current combination therapy of pegylated-interferon-α and ribavirin gives a 70-80% sustained virological response against most genotypes, but only 40% against genotype 1. Finding a more effective anti-HCV treatment has been a major objective of pharmaceutical companies for the past decade. In an effort to find a "Magic Bullet", a number of the enzyme activities of HCV have been targeted. The approach undertaken at Schering-Plough Research Institute has been to use structural information to assist the more traditional assay-based methods to move compounds that inhibit viral enzyme activity from a "Hit" to a "Lead" state, then through optimization into a clinical candidate. This research paradigm has resulted in an inhibitor of the HCV NS3 protease entering Phase III Clinical studies. An overview of this Structure-Based Drug Design process will be presented.

The Potential of Orally Presented Mistletoe Lectins in Cancer Therapy.

PRYME IF

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Background: Hitherto mistletoe preparations have been essentially administered by subcutaneous (s.c.) injection. This route, however, has clear limitations. Evidence now shows that a preparation containing mistletoe lectins (MLs) can be presented orally achieving the same results as s.c. administration. Being heavily glycosylated MLs can be taken orally since they are both resistant to low pH in the stomach and are unaffected by proteolytic enzymes.
Methods: Experiments were performed on female NMRI mice. Mice were initially maintained on a standard pellet diet with free access to water. Groups of five mice were prefed for 3 days on a Lactalbumin based, semi-synthetic diet (La). All mice were then injected s.c. with 2 x 10^6 Krebs II Non-Hodgkin´s Lymphoma (NHL) tumour cells. One group was switched to an ML-1 diet (lectin concentration : 1.7mg/ kg diet) a second (control) group was continued on the La diet. After 11 days animals were sacrificed and solid s.c. tumours were excised, weighed and prepared for histological examination.
Results: Following binding to specific receptors ML-1 induces a biological response. Immunomodulatory effects are initiated resulting in both activation and an increase in the population of natural killer cells. Many NHL cells enter the apoptotic pathway. An anti-angiogenic response severely curtails tumour growth. An interesting property of ML-1 is modulation of tumour characteristics seen as an increased level of differentiation.
Conclusions: In addition to the effects mentioned in the Results section ML can exert a cytotoxic effect on sensitive cells (e.g. cancer cells) bearing surface receptors to which the B-chain can bind. On internalization of the A-chain, and its activation, a RIP effect (ribosome-inactivating protein) is exerted leading to an induction of apoptosis, cumulating in tumour cell death. An advantage of using the oral route is that MLs can come into direct contact with tumours that are localized in the oral cavity, oesophagus or gastro-intestinal tract and thus be able to exert a direct cytotoxic effect on tumour cells. A further advantage of using the oral route is that injection is avoided and the involvement of health personnel is thus unecessary. It is proposed that the use of an oral mistletoe lectin-containing preparation would provide an excellent alternative, or supplement, to conventional forms of cancer treatment.

Virus-Like Particles As Magic Bullets For Immune System

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The development of genetic engineering techniques in the 1970s offered a broad range of applications, which were immediately followed by the expression of viral and non-viral genes in efficient heterologous expression systems, first of all, in bacteria and yeast. Special attention has been devoted to the synthesis of viral structural proteins as constituents of viral capsids and envelopes with their subsequent spontaneous self-assembly into correctly organized virus-like particles (VLPs). Food and Drug Administration's (FDA)-approved vaccines against hepatitis B and human papilloma viruses represent genetically engineered VLPs generated in heterologous expression systems. VLP-based vaccines are also being developed against malaria, HIV/AIDS, hepatitis C, human and avian influenza, as well as against many other infectious and non-infectious diseases. Moreover, VLPs from almost all classes of viruses are being evaluated now or have just been adopted to use as a scaffold for presentation of foreign immunological epitopes on their surface. VLP technologies possess obvious advantages for generation of safe and efficacious prophylactic and therapeutic vaccines. First, the repetitive antigenic structure of VLPs makes them highly immunogenic. Second, chimeric VLPs are lacking viral genomes or genes and are non-infectious, although they are mimicking infectious viruses in their structural and immunological features. Third, VLPs are generated by highly efficient heterologous expression of the cloned viral structural genes with subsequent quantitative *in vivo* or *in vitro* self-assembly of their products. Fourth, VLPs can be obtained by simple and efficient purification procedures. A broad range of viral structural proteins is able to form autologous VLPs consisting solely of structural protein(s) of the target virus. Many of them have been tested successfully for the construction of chimeric VLPs retaining their VLP-forming ability, but carrying foreign epitopes. VLP technologies allow the generation of (1) uniform chimeric VLPs consisting of identical fusion protein subunits, (2) mosaic VLPs consisting of carrier and fusion protein subunits, and (3) pseudotyped VLPs consisting of non-fused autologous and foreign proteins. VLPs can be used for a broad range of applications, including nanotechnology, but first of all for vaccine development, as magic bullets for immune system.

Elucidating Inhibitor-enzyme Interaction of Highly Potent Non-nucleoside Reverse Transcriptase Inhibitors Active Against Wild-type and Mutant HIV-1 Strains: Computer-aided Molecular Design Approaches

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Background: Because of the drug resistance, the treatment for AIDS still remains the worldwide medical problem. Efavirenz, a second-generation NNRTI, has recently been approved to treat HIV-1 infection with the high inhibitory activity. However, the efficacy of efavirenz is significantly diminished by the rapid drug-resistance mutations of HIV-1 RT, in particular the K103N. Thus, it is necessary to find new and more effective inhibitors remaining active across these virus mutations. To gain an insight into the potential binding orientation and the interaction of efavirenz derivatives with HIV-1 RT, docking studies, 3D-QSAR and quantum chemical calculations (QCC) were performed for efavirenz derivatives in the WT and K103N HIV-1 RT binding pocket.
Methods: The starting geometry of efavirenz was taken from X-ray crystallographic data. Efavirenz compounds were built and fully optimized at HF/3-21G level. Docking studies were carried out for efavirenz derivatives in the WT and K103N HIV-1 RT binding pocket. Based on the docked binding conformations, 3D-QSAR methods using CoMFA and CoMSIA were applied. The individual interaction between some derivatives and the surrounding amino acid in the WT and K103N binding pocket were investigated using QCC at single point calculation of MP2/6-31G(d) levels of theory.
Results: The potential binding orientation of the inhibitors in the binding pockets could be identified, by using docking studies. The docking results provide additional insight into essential inhibitor-enzyme interactions for different types of wild type and mutant type of HIV-1 RT. Based on the docking conformations, the reliable and predictive CoMFA and CoMSIA models of efavirenz derivatives for the WT and K103N RT inhibition were derived. The models are successfully used to discriminate between the structural requirements for WT and K103N inhibitory activities. Moreover, the interaction energy trend calculated from QCC of the inhibitors and individual amino acid residues in the binding pockets is informative to highlight particular ligand-receptor interaction in molecular level. The results derived from all approaches validate each other and agree well with the ligand-receptor complex interaction derived from the X-ray crystallographic data.
Conclusions: The molecular docking calculations, 3D-QSAR analyses and QCC were successfully combined to investigate the interaction and the relationship between structural requirements of efavirenz derivatives for WT and K103N HIV-1 RT. Consequently, the obtained results enable to provide beneficial guidelines to design novel compounds with higher anti-HIV–1 RT activities against WT and K103N RT.

Molecularly Imprinted Polymers (MIPs) for the Drug Targeting

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Background: Specific molecular recognition is a fundamental requirement of living systems and, through millions of years and countless rounds of evolutionary optimization, biology has become a master of the art.
At the cellular and sub-cellular level the fundamental processes of life, information transfer and reaction catalysis, rely on the specific interaction of low molecular weight molecules with macromolecular hosts. In the majority of such events the macromolecule is a protein.
Processes as diverse as neural transmittance, respiration, immune defence, cellular differentiation and nutrition all rely on the basic principle of specific molecular recognition.
It is therefore not surprising that scientists have invested huge amounts of time and effort, initially into harnessing the potential of biological molecular recognition, antibodies and enzymes, and more recently in trying to mimic these properties in synthetic materials. One of the most promising of these ones is molecular imprinting.
Molecular imprinting is a very useful technique for incorporating specific substrate recognition sites into polymers. The molecular recognition characteristics of these polymers are attributed to the complementary size, shape, and binding sites imparted to the polymers by the template molecules
The concept of molecular imprinting has a long history dating back to the early 1930s. However, the preparation of organic polymers with molecular recognition was first reported only in 1972 ,initiating the molecular imprinting technology as it is known today. Molecular imprinting has now become an established method and has also been applied in the areas of synthetic chemistry and analytical chemistry. MIPs have been used as chromatographic stationary phases for enantiomeric separations , and for solid-phase extraction , catalysis and sensor design, as well as for protein separation, as receptor, antibody and enzyme mimics and recently Drug Delivery Systems too .
But it is perhaps in the area of drug delivery, in particular “intelligent drug release” and “magic bullet” drug targeting, that significant future opportunities lie.
Although relatively few studies have been reported in the literature about the intelligent drug delivery and targeting, they represent an important starting point for the development of new generations of intelligent and selfregulated drug delivery systems.

Steroid Sulfatase Inhibitors – Novel Therapeutic Agents for Hormone Dependent Cancers

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Background: Inhibition of steroid sulfatase (STS), the enzyme responsible for the hydrolysis of steroid sulfates, represents a potential novel treatment for postmenopausal women with hormone-dependent breast cancer. Estrone and DHEA are formed by the sulfatase pathway and can be converted to steroids (estradiol and androstenediol, respectively), which have potent estrogenic properties.
Methods: STX64, a tricyclic coumarin-based sulfamate that irreversibly inhibits STS activity, was selected for the first in class Phase I trial of a STS inhibitor in postmenopausal women with breast cancer. STX64 was administered orally (nine patients at 5mg and five patients at 20mg) as an initial dose followed 1 week later by 3 x 2 weekly cycles, with each cycle comprising daily dosing for 5 days followed by 9 days off treatment. Blood and tumor tissue samples were collected for the assessment of STS activity and serum was obtained for steroid hormone measurements before and after treatment.
Results: The median inhibition of STS activity by STX64 was 98% in lymphocytes and 99% in breast tumor tissue at the end of the 5-day dosing period. Serum concentrations of estrone, estradiol, androstenediol and DHEA all decreased significantly from pretreatment levels. Unexpectedly, androstenedione and testosterone concentrations also decreased. Four patients, all of whom had previously progressed on aromatase inhibitors, showed evidence of stable disease for 2.75 to 7 months. The drug was well tolerated.
Conclusions: STX64 is a potent, well tolerated STS inhibitor which causes significant decreases in serum concentrations of estrogenic steroids. Conversion of second generation STS inhibitors to Magic Bullets will be highlighted.

Synthesis Of Fluorescent Heteroaromatic Compounds Using Dehydroamino Acids As Building Blocks, Studies Of DNA And Biomembranes Interactions. Evaluation Of Antiproliferative Effects On Tumor Cell Lines

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Background: The synthesis of new anticancer agents is an important goal. Aims: 1) To synthesize new fluorescent compounds, a 3-(dibenzothien-4-yl)indole (**1**), a 3-(benzothien-3-yl)benzothieno[2,3-*b*]pyrrole (**2**) and a 3-(benzothien-2-yl)benzothieno[3,2-*b*]pyrrole (**3**). 2) To study their interaction with DNA and liposomes. 3) To evaluate their effects in tumor cell lines.
Methods:. Absorption and fluorescence spectroscopies were used to study their photophysical properties in different solvents and their interactions with salmon sperm ds-DNA including fluorescence quenching experiments with iodide ion. Their interaction with liposomes of dipalmitoyl phosphatidylcoline (DPPC) prepared by injection, was studied by fluorescence. The antiproliferative effects on tumor cell lines of breast adenocarcinoma (MCF-7), glioblastoma (SF-268) and non-small cell lung cancer (NCI-H460) were evaluated after a continuous exposure of 48h, using the protein-binding dye sulforhodamine B. Results represents means ± SEM of 3 exp. performed in duplicate.
Results: Compounds **1**-**3** were synthesized in good yields. In the fluorescence spectra a red shift in the λ_{em} (nm) is observed from apolar to polar solvents. In the fluorescence spectra using increasing [DNA]/[compound] ratios an increase in the emission intensity is observed. The fraction of molecules accessible to iodide ion was very low. In DPPC liposomes (25 ^{o}C) the emission spectra are very similar to the ones in cyclohexane. The results of the antiproliferative effects are shown below.

Compound	GI_{50} (μM)		
	MCF-7	SF-268	NCI-H460
1	11.00±0.60	17.0±1.20	12.70±1.50
2	7.88±0.08	7.85±1.26	14.13±1.73
3	19.10±11.50	38.70±8.90	3.90±0.30

Conclusions: 1) The preferred mode of binding with DNA is the intercalation. 3) Their location in liposomes of DPPC is the hydrophobic region. 4) A good to high inhibitory effect on the growth of the tested cell lines was observed. Compound **3** shows a high specificity for the NCI-H460 cell line.
Thanks are due to the Fundação para a Ciência e Tecnologia (FCT, Portugal) and FEDER to financial support through the research centres, the research project POCI/59407/2004 and pos-Doc grants attributed to A.S.Abreu (SFRH/BPD/24548/2005) and to L.V.-S. (SFRH/BPD/29112/2006).

Addition Of Local Antiseptic Spray To Antibiotic Regime Reduces The Incidence Of Stomal Infection Following Percutaneous Endoscopic Gastrostomy (PEG) – A Randomised Controlled

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Background: Stomal infection (SI) following PEG is commonly due to bacteria coming from oro-pharynx and or skin surface. We hypothesise that by combining parenteral antibiotic with local anti-septic spray reduces the incidence of stomal infection by reducing the bioburden at the skin surface.
Aim: To study the effectiveness of local antiseptic spray with or without 3 dose parenteral antibiotic in the prevention of SI following PEG.
Methods: 96 patients randomised into 3 groups. Group A – intravenous (IV) Cefuroxime 750 mgs just before the procedure followed by 2 further doses 8 hourly. Group B – single application of Povidone – Iodine local antiseptic spray (Betadine). Group C –combination of A & B. Stomal site examined at midweek (3^{rd}/4^{th} day) and on day 7 for evidence of SI using a scoring system. Fisher's Exact Test used for analysis of primary end point [SI at midweek (MW) and end of week (EOW) 1]. Logistic regression (LR) models used to consider effects of age, sex, diabetes, acid suppressants and steroid therapy on outcome.
Results: Total 96 patients. Group A (n=34) M:F 18:16, mean age (MA) 74. Group B (n=28) M:F 15:13, MA 72. Group C (n=34) M:F 17:17, MA 74. Indications in A,B,C were broadly comparable. SI at MW in A,B,C were 6 %, 32 %, 9 % and at EOW 1 were 32 %, 32 % & 3 % respectively. SI at MW higher in B (32 %) with 6 % in A & 9 % in C (p=0.0114) and at EOW 1 lower in C (3%) with 32% each in A & B (P=0.0013). Cumulative infections (n) at EOW 1 in A, B, C overall were 11, 12, 3 with significant reduction in S I in C (p=0.003). No significant difference in numbers given antibiotics for other indications between the 3 groups (p=0.363). LR showed only diabetes to have a significant effect on SI (OR at MW 33.34, 95% ci: 4.33 – 256.7)
Conclusion: Cefuroxime + Betadine spray significantly reduces both midweek and end of week 1 stomal infection following PEG. Betadine spray on its own does not reduce stomal infection at midweek and end of week 1. Prophylaxis with 3 doses of IV Cefuroxime reduces stomal infection at midweek but not at end of week 1.

Synthesis And Biological Evaluation Of Some New 2-Cinnamamidobenzamides As Potential Antagonists Of The HDM2-P53 Protein-Protein Interactions

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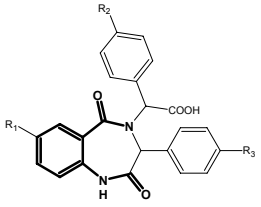
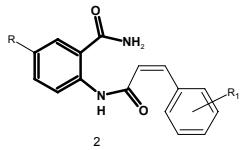
HDM2 is a negative regulator of the tumor suppressor p53. HDM2-p53 interaction maintains p53 in the "off" position and supports its degradation. Because HDM2 overexpressed in many cancers that retain wild-type p53, small molecules that target HDM2 are useful candidates to obtain pharmaceutically acceptable drugs. Among these, substituted 1,4-benzodiazepin-2,5-diones 1 are α-helix mimetic antagonists of the HDM2-p53 protein-protein interaction [1].
We synthesized 2-cinnamammidobenzamides 2 structurally correlated to compounds 1 with the aim to ascertain if they could be inhibitors of HDM2-p53 interaction.



1



2

Compounds 2 were preliminary tested for their in vitro antiproliferative activity against p53 mutant K562 (human chronic myelogenous leukaemia) and p53 overexpressed HBT-144 (choriocarcinom placenta) cell lines. Some 2-cinnamamidobenzamides 2 posses a significant antiproliferative activity against both K562 and HBT-144 cell lines and, among these the most active was the 5-iodo-2-cinamamidobenzamide (R=I, R_1=H) with an IC50 of 0.57 μM and 0.28 μM against K562 and HBT-144 respectively. Moreover, the presence of substitutions in the cinnamamido moiety lowered the antiproliferative activity only against the K562 cell line. Infact, 5-bromo-2-cinnamamido-4'-chlorobenzamide (R=Br, R_1=Cl) showed an antiproliferative activity at 10 μM of 18% and 80% against K562 and HTB-144 cell lines respectively. Biological studies to verify if the HMD2-p53 interaction is the real molecular target of compounds 2 are in progress.
References: Cummings, M.D.; Schubert, C.; Parks, D.J.; Calvo, V.L.; La France L.V.; Lattanze, J.; Milkiewicz, K.L., Tinbao Lu Chem. Biol. Drug Des. 2006, 67, 201-205.

Breaching The Barriers Of The Brain: From Physics To Cures

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Background: Getting the right dose to the right target is critical to any form of delivery for brain therapies, and the blood-brain barrier is central to regulating this. In individuals, diseases and therapies modify key factors of molecular motion, thus complicating the problem. We model the physics of brain to predict response to new protocols, devices, and therapeutics. Delivery methods range from systemic delivery of small molecules, to direct injection of large ones into tissue.
Aims: 1) Model key determinants of molecular transport within and into brain tissue using inputs from *in-vivo* imaging. 2) From this, obtain time-and space-dependent maps of molecular motion in response to therapeutic interventions. 3) Use this tool to develop and analyze *individualized* optimal delivery protocols and devices for application by a range of health care providers as well as drug developers.
Methods: We developed transport models, solution methods, and estimates of key parameters in the equations, for *individuals* from *in-vivo* imaging. Interstitial transport is validated using radiological contrast agents, and transcapillary transport via microdialyis. Direct delivery in tissue was studied in six pigs with concentration of a contrast agent measured with new quantitative imaging, and in seven humans using radioactive iodine. Monkey studies are ongoing. Systemic delivery is studied using microdialysis in humans.
Results: 1) Modeling and display suggest alterations of widely accepted therapy protocols and device placement, *e.g.* in brain cancer, we propose earlier administration of temodar (systemic) and entirely new catheters and placement (peritumoral infusions). 2) The extracellular spaces can expand to more than double their resting value in response to disease-induced edema or therapeutic infusions, having a major impact on flow of fluid and particulates in brain. 3) Determinants of transport can be quantified by specialized, but clinically acceptable, *in-vivo* imaging.
Conclusions: 1) Patient-specific estimation of distribution of therapeutics can be valuable in maximizing the chance of success of clinical trials, and in therapeutic outcome. 2) Inadequate dosing is a serious issue in therapeutics that requires collaboration of diverse disciplines and technologies. 3) Understanding the physics (poroelasticity, microhydrodynamics) of the brain is an important companion to pharmacology in getting drugs to desired locations.
Authors' disclosure statement: The experiments were conducted by collaborators at the Virginia Commonwealth University (pigs), Duke University (humans with radioactive markers), the Johns Hopkins University (humans with microdialysis), and the University of California in San Francisco (monkeys). The collaborators will be named in the talk. The work was supported in part by BrainLAB AG, which has a financial interest in the software developed in the course of the studies.

Identification Of Novel Hepatitis C Virus Polymerase NS5B Inhibitors Through Structure-Based Virtual Screening And 3-D QSAR Studies

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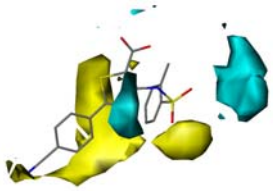
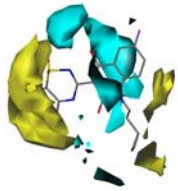
Background: Although a great deal of research has beeen focused on the developement of anti-HCV agents, to date no vaccine is available and there is no effective therapy for all genotypes. Thus, there is an urgent need to identify and develop HCV-specific antiviral agents to improve the effectiveness of actual HCV therapy.
Nonstructural protein 5B (NS5B), a 66 Kda RNA-dependent RNA polymerase (RdRp) has attracted the attention of medicinal chemists as a target for drug development since it plays a pivotal role in HCV replication.
Methods: Here we present the development of structure-based 3-D QSAR models for inhibitors binding either at the thumb or the palm NS5B allosteric sites. The final models proved to be statistically robust showing q^2 and r^2 values in the range of 0.5-0.9. The use of external test sets showed good predictive abilities of the 3-D QSAR models.
Through either ligand based (Surflex) or structure based (Autodock) molecular alignment the NCI Diversity Set was then virtually screened and the result were externally scored with the 3-D QSAR models. For each allosteric site, the first 20 molecules predicted more active were selected for biological assay against NS5B.
Results: Preliminary biological data proved that among the selected compounds three derivatives showed to be effectively active against NS5B at a fixed dose of 100 μM with percentage of enzyme inhibition in the rang of 60-70%. Details of computational and virtual screening procedures will be reported along with further biological investigations



Thum Binding Site



Palm Binding Site

The Immunosupressive Drugs- Cyclosporin A, FK506 And Rapamycin Modulate The Functional Expression Of The Na^+-Ca^{2+} Exchangers In An Isoform Specific Manner

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Background: Treatment of organ transplant patients with immunosupressive drugs leads to complications such as hypertension, nephrotoxicity, neurological symptoms and bone loss that can be linked to impaired cell Ca^{2+}. The Na^+-Ca^{2+} exchanger is a major cell Ca^{2+} regulating protein encoded by three genes: *NCX1*, *NCX2* and *NCX3*. NCX1 protein is expressed ubiquitously and NCX2 and NCX3 are expressed almost exclusively in the brain. We have investigated the modulation of NCX expression by the immunosupressive drugs Cyclosporin A, FK506 and Rapamycin and the non-immunosupressive PSC833. The drugs were added to the cultured cells.
Methods: Functional expression of NCX protein was measured in transfected HEK 293 cells and non-transfected-NCX1-expressing H9c2 cells. Na^+ dependent Ca^{2+} fluxes, surface and total NCX protein expression were determined in parallel by FACS analysis, surface biotinylation, Western analysis and quantitative PCR. siRNA targeting Cyclophilin A was used for its knock down. All measurements were done in triplicates and repeated at least 5-7 times.
Results: Treatment of *NCX1, NCX2 and NCX3*-transfected HEK 293 cells with Cyclosporin A and PSC833 results in down regulation of surface expression and transport activity of the protein without a decrease in expression of cell NCX protein. But whereas CsA had no effect on total cell NCX1 protein expression, PSC833 reduced mRNA and cell protein expression of NCX2 and NCX3. FK506 had no effect on NCX1 expression yet it down-regulated NCX2 and NCX3 surface expression and transport activity without any significant effect on cell NCX expression. Rapamycin had no effect on NCX2 and NCX3 protein expression yet it reduced NCX2 and NCX3 transport activity. Knock-down of Cyclophilin A modulated NCX1 expression and the effect of Cyclosporin A.
Conclusions: Since all the experimental conditions in our studies were identical, presumably the different drug response is related to structural differences between NCX isoforms. Expression of NCX genes is tissue specific.

Culture, Susceptibility Testing And Genotyping Of *Mycobacterium Tuberculosis* Isolated From Tuberculosis Patient In Bangladesh

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Background: It is essential to study the magnitude of the burden of tuberculosis (TB) in order to control the disease efficiently. Aims: 1) To isolate *M. tuberculosis* from sputum samples of TB patients, 2) Susceptibility testing of isolates and 3) To record circulating phylogenetic clades of *M. tuberculosis*.
Methods: *M. tuberculosis* was isolated from the pulmonary TB patients of selected rural and urban areas of Bangladesh. Conventional method was followed to isolate the pathogen from sputum samples and to tests susceptibility with respect to first line anti-TB drugs. Strains were genotyped by spoligo typing technique. Phylogenetic clade designation was performed matching the spligo patterns with that available in the International Database: SpolDB4.
Results: This study-included susceptibility testing of 657 isolates. Resistance to isoniazid, rifampicin, streptomycin and ethambutol was 14 %, 6%, 45 % and 8 % respectively. Simultaneous resistance to isoniazid and rifampicin was detected in 6% of the isolates. Randomly selected 224 strains were genotyped by spoligo typing. One hundred and ninety three (86%) of 224 isolates were grouped into 31 clusters containing from 2 to 34 isolates and 31 pattern isolates (14%) were unique. The comparison of spoligopatterns with SpolDB4 indicares that 75% of *M. tuberculosis* population of this study composed of Principal Genetic Group 1 (PGG1) having clades like; East African Indian (EAI, 44%), Beijing (15%), and the Central-Asian (CAS, 15%). The remaining 25% of the isolates belonged to PGG 2 and 3 having Latin-American-Mediterranean (LAM) clade as predominant. A new pttern signature was detected in 49 out of 224 isolates within the clade EAI (EAI 6 BGD1) and was named Matlab type after the name of the field site where it was isolated for the first itme.
Conclusion: Drug resistance was significantly higher among patients previously received anti-TB treatment. Besides, this study provides a first description of the genetic population structure of *M. tuberculosis* in Bangladesh, where TB patients are infected with a diverse and heterogeneous population of M. tuberculosis without predominance of a single genotype. The newly described Matlab types are suggestive of new South or South-East Asian-linked emerging genotypes.

The Herpes Simplex Virus (HSV) Vaccines: Old Problems, New Challenges

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Background: The HSV candidate vaccines tested until know were either purified subunit vaccines or recombinant envelope glycoproteins. In mice, guinea pigs and rabbits immunized with a classical subunit vaccine (Rajčáni, J et al.: Acta virol. 39, 1995, p.37-49), clear-cut protection against virus challenge and reduction of the extent of latency were demonstrated.
Methods: To compare our experimental HSV vaccines (based on the purified cell extract and on the recombinant gD polypeptide), Balb/c mice were immunized with the recombinant fusion protein gD1/313 (FpgD1/313, the ectodomain of gD1), with the non-pathogenic ANGpath gE-del virus, with the purified cell extract and with a plasmid (pcDNA3.1-gD) expressing gD1.
Results: High grade protection against virulent virus challenge was found after immunization with the pcDNA3.1-gD plasmid and with the gE-del virus. A medium grade, but still satisfactory protection was noted following immunization with the FpgD1/313 recombinant glycoprotein. Considerable response of peripheral blood leukocytes (PBL) in lymphocyte transformation test (LTT) was found in the mice immunized with FpgD1/313, with pcDNA3.1-gD plasmid as well as with live ANGpathE-3-3 virus. *In vitro*, secretion of Th 1 (TNF, IFN-γ and IL-2) and Th 2 (IL-4 and IL-6) cytokines was followed in purified PBL as well as in splenocyte cultures coming from immunized and control animals. The leukocytes from FpgD1/313 immunized mice showed increased secretion of both, Th 1 and Th 2 cytokines. The secretion of IL-4 and TNF was high by PBL of FpgD1/313 immunized mice (as compared to mock-immunized animals); the splenocytes from FpgD1/313 immunized mice showed extensive IL-4 production and a slightly elevated IL-6 synthesis.
Conclusions: The classical purified subunit vaccine (especially in combination with a novel adjuvant), might be a more powerful immunogen than a single recombinant glycoprotein.
Authors' disclosure statement. Introduction of new adjuvants, which shift the cytokine production towards the stimulation of helper T-cells indicates a promising development. Even when the immunotherapeutic use of HSV vaccine is effective only partially, it still might represent an alternative to chronic chemotherapy of recurrent labial and/or genital herpes.

Prevalence And Characteristics Of Verotoxigenic Producing Escherichia Coli O157:H7 Isolated From Goats And Cattle Carcasses In Tanzania

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The prevalence of Verotoxigenic producing Escherichia coli (VTEC) in cattle and goats carcasses were investigated between September 2002 and December to June 2003 by cultural and immunomagnetic separation technique. A total of 167 Escherichia coli colonies from carcasses of cattle (300), and goat (263), from Morogoro and Dar-es-salaam were isolated in this study. TEC O157 strains were recovered from 17 (5.67%) cattle carcasses and none from goats. Of 167 E.coli strains, 17 were grouped into sorbitol non-fermenting and glucuronide negative and 29 strains were sorbitol positive and glucuronide positive. The remaining 38 were sorbitol negative and glucuronide positive.V Using Reversed passive latex agglutination kit from Denka Japan indicated that all isolates produced verotoxin. Further characterization of the VTEC isolates showed that 1(4%) of the bovine VTEC strains was positive only for stx1. Stx2 gene alone was detected in 4(20%) of bovine isolate. Both stx1 and stx2 gene were present in one (4%) of bovine isolates. Eae A was detected in 4 (20) of bovine isolates. Stx 1, stx2 eae A and Ehly A were present in one (4%) bovine isolates. Other bacterial agents such as Pseudomonas spp, Proteus spp and coliforms were also isolated. The VTEC O157 isolates were resistance to gentamicine, chloramphenicol, streptomycin, and amoxsylav. This study is the first attempt to investigate the prevalence of VTEC O157 in goats and cattle carcarsses in Tanzania. Cattle carcasses are contaminated with verotoxigenic Escherichia coli O157:H7 in this region.

Effectiveness Of Antibiotics In The Management Of Acute Exacerbations Of Chronic Obstructive Pulmonary Disease. Do Antibiotics Improve Patient Outcomes – Evidence To Date

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Background: True efficacy of antibiotic use in the treatment of acute exacerbations of chronic obstructive pulmonary disease (AECOPD) remains controversial. Most patients are treated with a broad-spectrum antibiotic during an exacerbation, and a number of controlled trials have shown their beneficial effects. However, some trials have shown a lack of positive effect with antibiotic therapy. Despite the many clinical trials and guidelines the role of antibiotics in the management of AECOPD remains a matter of debate.
Methods: We undertook a systematic review with meta-analysis of all available high quality randomised controlled trials. Trials were included in the review if an antibiotic was compared to placebo in patients with AECOPD. Trials were searched for on the Cochrane database, MEDLINE, EMBASE, Web of Science, key respiratory web sites, journals and also hand searching of journals.
Results: Eleven trials with 917 patients with moderate-to-severe COPD exacerbations were included in the review. Antibiotic therapy reduced relative risk (RR) of mortality by 77% (RR 0.23; 95%CI 0.10, 0.52), treatment failure by 53% (RR 0.47; 95%CI 0.36, 0.62) and sputum purulence by 44% (RR 0.56; 95%CI 0.41, 0.77). The number of patients required to be treated (NNT) with antibiotics to save one life was 8 (95%CI 6, 17) to avoid one treatment failure 3 (95%CI 2, 4) and to avoid one patient having purulent sputum was 8 (95%CI 5, 14). However, and as expected, antibiotic use increased the risk of diarrhoea (RR 2.86; 95%CI 1.06, 7.76).
Conclusions: Our results clearly show that in moderate-to-severe exacerbations of COPD associated with increased cough and sputum purulence, antibiotic therapy regardless of antibiotic choice, is efficacious in decreasing risk of mortality, treatment failure and sputum purulence. This review supports the use of antibiotics for patients with AECOPD who are moderately or severely ill. There should now be no doubt that antibiotic chemotherapy plays an important role in the management of patients with AECOPD.

Ciprofloxacin Resistance Profile In *Klebsiella Pneumoniae* Isolates During 2002-2007 In Paediatric Septicaemic Cases Of A Tertiary Care Hospital In India

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Ciprofloxacin was introduced in the late 1980s as an efficacious and safe drug. It's usage in India increased phenomenally in the period after the local outbreak of plasmid mediated chloramophenicol resistant *S. Typhi* in 1993, which resulted in the change of the drug of choice of typhoid fever from chloramphenicol to ciprofloxacin. The effectiveness of ciprofloxacin in numerous microbes and significant reduction in its cost has led to its rampant empiric use in many clinical conditions. The current study is to assess the trends of ciprofloxacin resistance in *Klebsiella pneumoniae*. More than five hundred and fifty isolates of *Klebsiella Pneumoniae* were isolated from septicaemic cases admitted in Kalawati children hospital during 2002-2007. The age of the patients varied between newborn to 14 years. The isolates were identified by standard microbiological (including biochemical) techniques. The resistance profile of the isolates was analyzed by antibiotic disc diffusion technique. The percentage of strains resistant annually to ciprofloxacin were calculated. The ciprofloxacin resistant isolates rose from 26.9% in 2002 to 63.8% in 2007. This resistance to ciprofloxacin is due to multiple chromosomal mutations and is associated with drug resistance to other antimicrobials. Thus, a rational and judicious use of ciprofloxacin is necessary, besides efforts to prevent spread of ciprofloxacin resistant *K. pneumoniae* strains in the hospital.

Polymyxin B Sulphate: A Brief Overview

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Background: Polymyxin B sulphate, an antibiotic discovered in 1947, is used in the treatment of infections caused by gram-negative bacteria, particularly the *Pseudomonas aeruginosa* and *Escherichia coli*. They are decapeptides with antimicrobial spectrum that includes gram-negative bacteria, prominently *Pseudomonas aeruginosa*. Many questions remain unanswered about polymyxins since most of the research was carried out before the 1980s and the methods for evaluation of the antibiotic were not as advanced as today. Therefore, a detailed investigation on the polymyxin production by *Bacillus polymyxa* is warranted.
Methods: Optimization of nutritional and physiological parameters for high yield of polymyxin B sulphate production was done at shake flask level. The process was scaled up to 10 L in a bioreactor. A process for purification of the antibiotic was developed.
Results: By optimization, a yield of 2 g/L of polymyxin B sulphate could be obtained. The process was scaled up and validation at 10 L level in a bioreactor. Downstream processing for isolation of the antibiotic involved steps including charcoal adsorption, chromatographic separation on Amberlite resin and solvent precipitation. A recovery of 33 % could be obtained.
Conclusions: A detailed study for optimized production of polymyxin B sulphate was done. The downstream process to isolate the antibiotic from the broth was developed.
Authors' disclosure statement: Some of the data being in the patenting stage cannot be presented

The Vienna Vaccine Safety Initiative (Vivi) – An International Scientific Forum Aiming To Promote Evidence-Based Vaccine Safety Research And Communication

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Background: Recent trials have confirmed the safety of recommended vaccines, but rumours surrounding the safety of human vaccines spread quickly via the international media causing confusion and anxiety among patients, parents and physicians alike.
Methods: In view of these concerns, The Vienna Vaccine Safety Initiative (ViVI; http://www.meduniwien.ac.at/kikli/vivi/) has been founded in 2008 as an international scientific forum aiming to promote evidence-based vaccine safety research and communication.
Results: Initial projects are dedicated to the role of the media in vaccine risk communication, and to perceptions of vaccine safety among vaccine recipients as well as providers. The goal is to generate feedback for the scientific community. Interim Results of a study assessing vaccine safety perceptions among parents in Vienna will be presented along with a survey of vaccine safety training and awareness among pediatricians in collaboration with the International Pediatric Association (IPA).
Conclusion: The Vienna Vaccine Safety Initiative has been formed to address the urgent need to promote vaccine safety training and research. Additional projects will serve to generate awareness of international vaccine safety standards in the public as well as pilot projects in collaboration with international vaccine safety experts from the developed and developing world. It is hoped that the activities of this new initiative will be able to generate concepts for evidence-based action towards accurate AEFI reporting while strengthening trust in vaccines.

Interfacing Cell's Membrane And Drugs' Mechanical Properties To Control Bioavailability: Application To Lipinski's 2nd Rule And Resistance To Drugs

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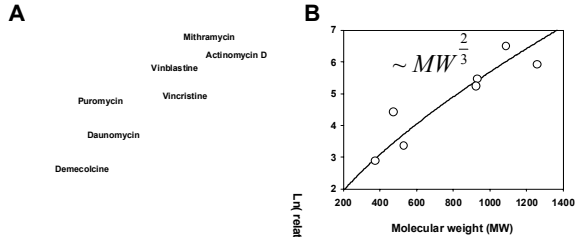
Background: An important bottleneck in drug discovery is determining the appropriate properties of a drug that facilitate its delivery. Lipinski has produced four rules that identify statistically the properties required for oral compounds to achieve maximum bioavailability (1). The second rule stipulates that drugs must have a molecular weight (MW) inferior to 500. As for drugs small enough their MW is proportional to their volume, Lipinski's 2nd rule suggests that drugs' volume is a limiting parameter when they cross membranes. Furthermore, as to be bioavailable, drugs must traverse lipid membranes; this rule suggests that a specific interaction occurs between cells' membrane and drugs' volume, affecting their bioavailability.
Methods: Based on this premise and using concepts associated with membrane biology and complex systems physics it is possible to demonstrate that, indeed, a *universal* MW cut off exists identical to Lipinski's 2nd rule.
Results: The *universal* MW cut off which defines Lipinski's 2nd "Law" is given by: $MW_c^{2/3}=(4/3\pi^{1/2})^{2/3}.(hRk_BT/8k_c)$, where MW_c, h, R, k_B, T, k_c are, respectively, the critical MW, membrane thickness, endocytic vesicle radius, Boltzmann's constant, temperature (in Kelvin) and membrane bending modulus. The theory provides a numerical value MW_c=456 very close to 500. Finally, this universal law regarding a MW cut off can also predict multi drug resistance levels. For example, relative resistance levels (selected using actinomycin D) to control in lung-derived cell line plotted as a function of drugs' MW (Fig.1A) agree well with the power law given by Lipinski's 2nd "Law" (Fig.1B; R^2=0.83).
Figure 1:



Conclusions: Given the formulation of Lipinski's 2nd "Law", it is now possible to modulate and predict how drugs cross the membrane of cells of either sensitive or resistant cells (not shown).
References: (1) Lipinski CA, Lombardo F, Dominy BW, and Feeney PJ. *Advanced drug delivery reviews* 46: 3-26, 2001.

Acetaminophen: The Global Pain Killing Magic Bullet Of The New Millennium

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Background: For decades now, acetaminophen (APAP) has enjoyed untainted popularity as the most effective and possibly the safest over-the-counter analgesic of the world. It has maintained its prestige by itself and in combination with several other drugs such as morphine, codeine, aspirin etc., which has mesmerized the field of medicine as the "Magic Bullet for Pain Management'. APAP uses COX1?/?COX2?/COX-3 as instruments to subdue pain, but unfortunately its safety and efficacy overshadows its potential to cause hepatotoxicity. Our laboratory has used APAP as a model toxin for decades to understand mechanisms of cell injury and cell death, and we were the first to report its potential to induce genomic injury and apoptotic cell death in the liver. APAP is unique because of its ability to communicate with various macromolecules and all the cellular components (membrane, ER, mitochondria, nucleus). CYP450-mediated biotransformation of APAP produces a highly reactive intermediate, NAPQI, and oxyradicals such as ROS, RNS & ROS-RNS hybrids. Concerted actions of all these dramatically deplete intracellular antioxidants, induce massive oxidative stress and provide a platform to initiate molecular changes that orchestrate various forms of cell death in the liver.
Methods: This study (♂ICR mice) was designed to explore some of the molecular mechanisms after exposure to toxic doses of APAP (500 mg/Kg, ip) for 0 - 24 hours. Livers were analyzed for tissue biochemistry, ultrastructural changes & differential expression of pro- and antiapoptotic genes.
Results: APAP induced massive liver injury (↑ALT) coupled with massive oxidative stress (↑lipid peroxidation) and genomic injury (↑DNA fragmentation). Gene expression studies revealed considerably reduced anti-apoptotic genes (bcl-2, bcl-XL) and elevated levels of pro-apoptotic genes (bcl-Xs, p53). Ultrastructural studies revealed megamitochondria formation and evidence of cytochrome c leakage a unique hallmark of mitochondrial route of apoptosis.
Conclusion: In addition to the classic apoptotic markers, this in vivo study clearly suggests APAP's ability to use the mitochondrial cytochrome c-release signaling system as a unique mechanism to propel this cell suicidal process. Since this pathway is maneuverable, it may have tremendous therapeutic implications in liver injury management.

Computational Investigation Of Infectious Disease Mechanisms: From HIV-1 Virus Inhibition To Bacterial Resistance To Antibiotics

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Computer simulations have become an invaluable tool to study at atomistic level the dynamical behavior of biomolecules inside cells and to predict how they might react to the presence of prospective drugs. In this seminar, recent applications of molecular modeling and molecular dynamics simulations to the understanding of the functional activity of proteins involved in relevant infectious diseases are presented. Specifically several aspects of HIV-1 protease and HIV-1 integrase, two proteins encoded HIV-1 virus, will be discussed. In addition, new insights on the hydrolysis mechanism of penicillin-like antibiotic, such as cefotaxime, by beta-lactamases and the onset of resistance to beta-lactamase inhibitors, like clavulanic acid and tazobactam, will be reported.

Lapatinib: New Expectations

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In the recent years there has been substantial improvement in the management of Her2Neu overexpressing breast cancer. The addition of trastuzumab, a humanized anti Her2 monoclonal antibody to the treatment of both metastatic and early disease has resulted in impressive amelioration of Disease Free survival and overall survival for these patients. However a significant number of Her2Neu overexpressing tumors have either primary resistance or develop it after response to trastuzumab. The mechanisms of resistance are varied and are the topic of extensive research, but it is clear that new molecules are needed for the management of refractory cases.
Lapatinib is a small, orally administered, reversible dual Erb 1 and Her2 inhibitor. It is a tyrosine kinase, which inhibits Erb phosphorylation and survivin thus inducing apoptosis. Lapatinib inhibits downstream signaling from the hetero-and homo-dimerization of EGFR, Erb2 and possibly other Erb family members, thus preventing or delaying the development of resistance.
Original phase I studies showed that lapatinib is active in nearly pretreated Her2 positive metastatic breast cancer with a median response duration of 5.5 months. Most frequent drug related event included diarrhea (42%) rash (31%) nausea (13%) pruritus and fatigue (10%). The development of rash did not seem to correlate with response. The optimally tolerated dose was 1500mg/day. Subsequent phase II trials at that dose level yielded a response rate of 4-8% in heavily pretreated patients but in the first line therapy of FISH positive patients the ORR was 28%.
The next study was a randomized phase III trial in Her2 positive pretreated patients who received either capecitabine 2500mg/m^2/day or capecitabine (same dose) plus lapatinib 1250mg/day. In this study median time to progression and progression free survival were statistically significantly longer in the combination group. Furthermore some responses were seem in CNS disease. Additional studies in this area reveal modest responses in CNS metastasis.
Further ongoing studies of lapatinib efficacy include randomized trials in combination with other chemotherapy agents (such as the taxanes) combination with trastuzumab for complete Her2 signaling blockade and combinations with hormonal therapy (letrozole). Also ongoing are trials in the neoadjuvant adjuvant setting and in CNS disease. Extensive investigation into the mechanism of resistance to Her2 blockade is also in progress specifically into molecular determinants of resistance such as PTEN, IGF-1R and akt and into the best method for identification of responders.
Last but not least a clear grasp of the cardiac toxicity associated with lapatinib is necessary. Because erb2 signaling is important for cardiac function there were concerns that lapatinib had the potential of cardiac toxicity.
The incidence of symptomatic and asymptomatic decrease in LVEF among the 1674 patients treated with lapatinib in the phase III trial was only 1.3% and the drop was reversible but ongoing studies are in progress.
Finally, ongoing studies in other Her2 overexpressing solid tumors are also in progress.

Antimicrobial Peptides From Bacteria: Towards Novel Magic Bullet Strategies?

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Background: Probably the oldest and most widespread antimicrobial strategy in living organisms is the use of antimicrobial peptides. Bacteria secrete gene-encoded antimicrobial peptides they use for microbial competitions, which are termed bacteriocins. Bacteriocins from lactic acid bacteria and from enterobacteria (termed microcins) exert potent bactericidal activities with minimal inhibitory concentrations in the nanomolar range. In the context of urgent need for novel antibiotics, underlined by the increasing antibiotics-resistance problem, bacteriocins and microcins may offer new and promising strategies.
Results: Nisin, a 34-residue lantibiotic bacteriocin, which is currently used as a food preservative, exerts a potent antibacterial activity by binding to lipid II, the essential precursor of cell wall synthesis, thus forming stable pores in membrane bilayers [1]. Microcin E492, a hydrophobic and anionic 84-amino acid peptide endowed with a C-terminal siderophore post-translational modification, targets the inner membrane of sensible bacteria. Microcin J25, a 21-residue cyclic peptide with a unique "lasso" three-dimensional structure inhibits RNA polymerase. Despite their low structural homology and different killing mechanisms, these two microcins have a similar import pathway into sensible bacteria that uses siderophore receptors responsible for iron import in bacteria [2]. In order to identify the structural regions involved in the recognition/translocation step and the killing mechanism, we are studying the structure-function relationships for these microcins.
Conclusions: The mechanism of bactericidal activity used by microcins involves: i) recognition by outer membrane receptors used for vital functions in bacteria, which have been piratized by microcins; ii) translocation of the antibacterial peptide into the periplasm; iii) bacterial killing that involves either interaction with the inner membrane to form a toxic structure or passage through the inner membrane to interact with a cytoplasmic target. Microcins and bacteriocins exhibit complex, subtle and clever mechanisms of bactericidal activity that pave the way for the design of novel antibiotics.
References: Hsu STD *et al.* Nat Struct Mol Biol 2004, 11, 963-7. Duquesne S *et al.* Nat Prod Rep. 2007, 24, 708-34.

Divalproex-Valproate: Is The Discovery, Research & Development With This Molecule And Its' Formulations Complete?

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Background: Valproic acid (VPA) syrup entered the USA (1979) for epilepsy. Due to nausea, an enteric-coated, delayed-release (DR) formulation was developed. Extended-release (ER) forms of antiepileptic drugs should increase compliance and tolerability. Recognizing that DR was not sustained-release, divalproex-ER tablet was developed for once-daily (QD) administration.
Methods: Data from >12 published or ongoing studies were analyzed to distinguish kinetics of VPA and DR vs. ER (Abbott).
Results: AAN 2005_M98-937_Ann Pharmacother The absolute bioavailability of ER is 89%; AAPS 2003_M95-414_J Clin Phamacol 2004;44:737-42 & ASCPT 2003_Biopharm Drug Dispos 2004;25:345-52. 8-20% greater doses are needed to achieve equivalent VPA AUC. Food does not interfere with the ER absorption. A meta-review of ER studies shows superior tolerability vs DR. ER functional half-life is >24h in induced, 40h in non-induced patients. ER displays net-zero-order absorption, clearly distinct from other forms. ER is approved for QD use; not DR. A structured simulation shows marked fluctuation in VPA concentrations with QD DR and clinical toxicity & breakthrough seizures reported with pharmacy substitution of DR for ER. In the rat epilepsy model, total peak plasma VPA are not more efficacious vs continuous iv VPA; AUC is the relevant metric, giving impetus for ER use. Minimal variability in lot-to-lot mg potency (± 2-3%) exists. A prospective, placebo-controlled trial on the pharmacodynamic EEG effect of small VPA changes via constant infusion, with paroxysmal photosensitive response is underway.
Conclusions: 1. Divalproex-ER exhibits unique kinetics.AES 2004_Submitted Ther Drug Monit 2. ER development was complex & time-consuming. 3. Divalproex celebrates 25 years of use in the USA (30+ years use elsewhere in the world), yet clinicians are still learning about the clinical utility of VPA and the ER formulation.

Uncovering Tumor Systems Biology By Biomodulatory Therapy Strategies

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How can get structured therapies in metastatic cancer a source for detecting tumor-associated systems-biological processes as adjustable sizes available for biomodulatory therapies?
A therapy-derived methodological approach to explore tumor-associated systems biology should be explicated and developed by means of analyses of recently published biomodulatory therapy approaches introducing combined immunomodulatory, anti-inflammatory and angiostatic therapy in the treatment for advanced chemorefractory tumors of quite different origin. Biomodulatory therapy approaches in tumors intend to develop systems-terms that provide a basis for broadening therapy-relevant capacities by regulating biological systems processes for tumor control. Combined targeted therapies of tumor-associated wound healing mechanisms, namely inflammation and neoangiogenesis, have shown that – using an approach for understanding systems biology as adjustable size – we may break through the barrier of complexity of tumor-stroma-interactions in a therapeutically relevant way.

Digoxin plasma concentrations of four different dosing schedules commonly used in clinical settings.

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Background: The different therapeutic schedules used for the prescription of digoxin have little theoretical support and are based mainly on the patients clinical response. The aim of this study was to measure digoxin plasma levels in patients using four different prescription schedules.
Methods: A total of 175 patients were included in this study with previous written consent. Inclusion criteria: patients who had been prescribed Digoxin for at least 1 month prior to the study. Four groups of patients were studied.
Group I (n=56) digoxin 0.25 mg/day, from monday to friday.
Group II (n= 30) digoxin 0.25 mg/day, from monday to saturday.
Group III (n= 53) digoxin 0.25 mg/day continuously.
Group IV (n= 36) digoxin 0.125 mg/day continuously.
Plasma digoxin levels were measured in two consecutive mondays, before taking the daily dose of the drug, serum creatinine and creatinine clearance was calculated. Therapeutic plasma concentration range was set between 0.5 and 2 ng/ml. Digoxin was determined using immune analyses through a fluorimetric enzyme.

Results:

	Group I	Group II	Group III	Group IV
Total Patients	56	30	53	36
Age	65 ± 13	68 ± 11	61 ± 13	67 ± 12
Female %	68	63	57	68
Digoxin 1st (ng/ml)	1.14 ± 0.77	1.38 ± 0.57	1.62 ± 0.74	1.12 ± 0.40
Digoxin 2nd (ng/ml)	1.19 ± 0.88	1.42 ± 0.60	1.74 ± 0.76	1.15 ±0.48
Digoxin average (ng/ml)	1.15 ± 0.80	1.40 ± 0.55	1.68 ± 0.70	1.14 ± 0.43
Creatinin Clearance < 50 ml/min	34	33	23	36

93% in group I, 80% in group II, 75% in group III and 94% in group IV had therapeutic digoxin levels.
Low creatinine clearance, age over 65 and drug interactions were risk factors associated with supratherapeutic levels, mostly seen in group II and group III with 20% and 24% respectively.
Conclusions: 1) Digoxin plasma levels proportionally increase as the weekly dose increases 2) Similar therapeutic plasma levels are observed in group I, II and IV 3) High proportion of supratherapeutic levels are seen when Digoxin is used with Amiodarone and in patients with Creatinine clearence smaller than 50 mL/min.

Pathogenesis And Targeted Treatment Of *BCR-ABL* Negative Myeloproliferative Neoplasms

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The chronic myeloproliferative neoplasms (MPN) are clonal disorders characterized by excess proliferation and normal maturation of cells from one or more myeloid lineages. The most common MPN are chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). Rare subtypes include chronic eosinophilic leukaemia (CEL), chronic myelomonocytic leukemia (CMML) and systemic mastocytosis (SM). The constitutive activation of the BCR-ABL protein tyrosine kinase as consequence of the reciprocal translocation t(9;22) in CML has become the paradigm for molecular pathogenesis and targeted treatment with tyrosine kinase inhibitors, e.g. imatinib. Meanwhile, it has become evident that acquired constitutive activation of protein tyrosine kinases is also a central feature in the pathogenesis of *BCR-ABL* negative MPN. The most commonly involved genes are the receptor tyrosine kinases *PDGFRA*, *PDGFRB*, *FGFR1* or *c-KIT* and the non-receptor tyrosine kinases *JAK2* and *ABL*. Activation occurs as a consequence of specific point mutations or diverse fusion genes generated by chromosomal translocations, insertions or deletions. Mutant kinases are constitutively active in the absence of the natural ligands resulting in deregulation of hemopoiesis in a manner analogous to *BCR-ABL* in CML. The most common point mutation is *JAK2* V617F which is identified in most patients with PV and about 50% of patients with ET and PMF while a KIT D816V mutation is found in 80-90% of patients with SM. The most common fusion gene is *FIP1L1-PDGFRA* which is present in a substantial proportion of patients with CEL. Imatinib induces high response rates in patients associated with constitutive activation of PDGFR□, PDGFR□, ABL, and some KIT mutants. Other inhibitors under development are promising candidates for effective treatment of patients with constitutive activation of JAK2, FGFR1 and imatinib-resistant KIT mutants. The indolent clinical course of distinct subtypes, e.g. PV or ET, and the efficacy and safety of currently used therapies will necessitate that JAK2 inhibitors have a favourable toxicity profile in the short- and long-term. Important questions for future studies remain the unknown molecular pathogenesis of approximately 30% of MPN patients and the potential role of additional genetic events in the pathogenesis of MPN.

Some Aspects On Xenobiotic Metabolism, From Hepatic Function To Prospective Drugs

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Xenobiotic metabolising enzymes, mainly cytochrome P450, are regulated intrinsically. The influence of amino acid residue 374 on CYP2D6 mediated metabolism of metoprolol will be presented as an example. However, they are also affected by environmental factors like diseases, drugs or diet.
Stress influences drug metabolism. The influence of cold hypoxic preservation and normothermic reoxygenation of liver slices on CYP3A oxidation will be given. Stressors (fasting, restraint) lead to increased resistance to xenobiotics. Experimental arthritis reduces P450 activity. Toxic agents also affect drug metabolism. Since high levels of glucocorticoids appear during stress, the effect of corticosterone on drug metabolism is examined. It is concluded that augmented metabolic activity can be stimulated as response to need. Triamcinolone increases drug metabolism of arthritic rats. It also ameliorates the impaired metabolic activity due by toxic agents. P450 inducers, such as PCN and spironolactone restore the reduced enzyme activity caused by toxic compounds and decreases cholesterol in hypercholesterolemic rats.
PCN is a CYP inducer free of other activities. The effect of various pregnenolone 16α substituents on drug metabolism is studied. Since the 16α-CN is found to be the most potent, the effect of the position of CN in cyanopregnenolones on drug metabolism is reported.
Various drugs interact with P450. The examined interaction of analogues of diphenhydramine and cimetidine on P450 indicates structural features determining this action.
The ability of some non steroidal anti-inflammatory drugs to induce P450 and peroxisomal proliferation is examined, in order to investigate their interrelationships.
Although oxidations are the most common metabolic reactions, several compounds are reductively biotransformed. Microsomal reduction of metyrapone analogues is examined and applications for safer insecticides are suggested.
Food constituents influence drug metabolism. Guaiazulene is found to inhibit several CYPs and to protect from paracetamol hepatotoxicity. Vitamin E increases metabolic processes.
Finally, the effect of new antidyslipidemic morpholines acting as squalene synthase inhibitos and novel compounds combining antioxidant and anxiolytic structures on cytochrome P450 will be given.

Receptor Tyrosine Kinases As Therapeutic Targets In Malignant Glioma, Present Status And Application In China

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Background: Receptor tyrosine kinases (RTK) and related cellular signalling pathways are important targets for treatment of malignancies. As novel anti-cancer agents, small molecule RTK inhibitors inhibit the phosphorylation and activity of the protein kinases, leading to blockade of the receptor-mediated signaling pathways in tumour cells. However, thus far, the majority of protein kinase inhibitors lack clinical utility as single agents. The aim of the study is to investigate the cellular and molecular effect of RTK inhibitor imatinib mesylate in malignant glioma cells. In addition, we briefly review application of some of the RTK inhibitors in the same malignancy.
Methods: The expression of genes was analyzed by qRT-PCR and western blotting in malignant glioma cells. Proliferation was measured by MTT assays and drug synergy was assessed by Chou-Talalay method; Cell cycle and apoptosis were analyzed by flow cytometry and migration by monolayer migration assays. siRNA was applied to knockdown platelet-derived growth factor receptor-B (PDGFRB) expression in glioma cells. Multi-immunoblot was performed on Imatinib-treated and control cells.
Results: Imatinib treatment was lack of growth inhibition activity in glioma cells. The treatment was more effective in inhibiting the phosphorylation of Bcr-Abl in K562 cells than that of PDGFRB in glioma cells. Knockdown of PDGFR-B by siRNA showed similar responses on an Antibody microarray as in the case to imatinib treatment in U87 glioma cells. In addition, imatinib induced specific changes in downstream signalling pathways of the cells. Most prominently, continous activation of extracellular signal-regulated kinases (ERK1/2) signaling and its downstream effectors, e.g. dual phosphatases and immediate early genes (IEG), MKP1 and -3, and ribosomal protein S6 (rpS6) were stably increased or altered by both immunoblot and qRT-PCR analysis. Continous activation of ERK induced by imatinib treatment was related to S-phase re-entry in U87 cells.
Conclusions: 1) Imatinib treatment induced a complex and dynamic modulation of downstream signal transduction pathways in glioma cells; 2) Synergistic interaction of Imatinib with chemotherapy agents may be related to cell cycle control mechanisms and could be potentially important in a clinical setting.

Long-Term Sequential Deferiprone-Deferoxamine Versus Deferiprone Alone For Thalassaemia Major Patients: A Randomised Clinical Trial

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Background: Changes in chelation treatment and transfusion practices have improved the prognosis of thalassaemia major patients dramatically. Controversial results have so far been published and no trial with treatment duration longer than 18 months has been published.
Methods: This study is a long-term sequential DFP-DFO treatment versus DFP alone trial. The main aims were: variations on multiple determinations of serum ferritin values, mortality, adverse events, and costs. Multicentre randomised open-label tria. DFP at 75mg/kg, divided into 3 oral daily doses, for 4 days per week and DFO by subcutaneous infusion at 50mg/kg/day for the remaining 3 days per week versus DFP alone at 75mg/kg for 7 days. Five years follow-up.
Results: 275 consecutive thalassaemia patients were assessed for eligibility. 62 were excluded because of not meeting inclusion criteria or refusal to participate, leaving 213 patients for randomisation and intention-to-treat analysis. The coefficient of regression treatment–year shows a statistically significant serum ferritin reduction in sequential DFP-DFO patients compared with DFP alone patients (p=0.005). Kaplan-Meier survival curves for the two chelation treatments did not show any statistically significant difference (long-rank test, p=0.3145). Adverse events were comparable across groups.
Conclusions: Sequential DFP-DFO treatment compared with DFP alone significantly decreases serum ferritin concentration during treatment for 5 years without significant differences regarding mortality or adverse effects.
Authors' disclosure statement: Trial Registration: Clinical Trials NCT00733811

Neonatal Abstinence Syndrome And Cerebral Infarction Following Maternal Codeine Use During Pregnancy

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Background: Neonatal withdrawal from maternal drugs is common in many neonatal intensive care unitss. Codeine-containing cough preparations given to pregnant mothers can cause neonatal abstinence syndrome. Many women do not consider prescription cough syrups when asked about drug use. Maternal medication or illicit drug use, including opiates, has been identified as a cause of perinatal arterial stroke. Since codeine is an opiate with pharmacodynamic effects similar to morphine, it is reasonable to investigate if maternal codeine use can have effects on the fetus similar to other opiates, including cerebral infarction.
Methods: We present 3 cases of newborn infants with perinatal arterial stroke associated with in utero exposure to codeine. The first infant had infarction of the left middle and anterior cerebral arteries. Her mother received a codeine-containing cough medicine for an URI for 2 weeks prior to delivery. The second infant had infarction of the left occipito-temporal region. The mother had been hospitalized with pneumonia and received a codeine-containing cough medicine also for about 2 weeks prior to delivery. A third case has recently been added. None of the infants had other identifiable causes for cerebral infarction. All presented with seizures and signs of neonatal abstinence. All were eventually discharged to home.
Results: Perinatal arterial stroke can be a consequence of many illegal drugs, including opiates. Neonatal abstinence syndrome as a result of maternal codeine use has been documented, even in non-addicted mothers. Opiates have been shown to cause neonatal thrombocytosis, apoptosis in microglia and neurons and vasoconstriction in the placental circulation and middle cerebral arteries. It is reasonable to assume that codeine, may have similar effects on the fetus when administered to the pregnant mother.
Conclusion: Physicians should investigate maternal medication use, including codeine-containing cough preparations, when evaluating newborn infants with neonatal abstinence syndrome and/or evidence of cerebral infarction, particularly if the mother does not have a history of illegal drug use. This discussion raises questions about the safety of codeine-containing treatments and other “magic bullets” for pregnant women.

Vindesine And Razoxane, An Effective Drug Combination For Soft Tissue Sarcomas

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Background: Vindesine and Razoxane were shown to enhance the radiation response and to suppress distant metastasis in animal systems. Both drugs affect main steps of the metastatic cascade. During the past 15 years, these drugs were evaluated in translational research studies in a variety of soft tissue sarcomas [STS] with emphasis given to the radiation response and the dynamics of metastasis.
Methods: In a phase II study, 23 patients with unresectable (n=7) and oligometastatic STS (n=16) received a basic treatment with razoxane (2x125 mg daily) and vindesine (2 mg iV/ week) supported by radiotherapy and occasionally by surgery. Long-term treatment was intended in patients with metastatic disease. Thirty-six patients with comparable stages and prognostic parameters treated with contemporary chemotherapy served as non-randomised, retrospective controls.
In addition, the outcome of a small multicenter study on vascular soft tissue sarcomas is reported. This study was performed by the Austrian Society of Radio-Oncology, using the same treatment regimen and study endpoints.
Results: In the first study, the combination of razoxane+vindesine+radiotherapy led to 89% major responses (CR + PR). The median number of new metastases after 6 months was 0 (range, 0-40) and after 9 months likewise 0 (0-70). The corresponding numbers in the control group were 4.5 (range, 0-40) and 9 (0->100) [p<0.001]. The progression-free survival at 6 months was 74% in the study group and 23% in the controls. The median survival from the occurrence of the first metastasis or time of unresectability was 20+ months versus 9 months [p<0.001]. The combined treatment was well tolerated but normal tissue reactions were enhanced.
In the OEGRO study, 6 of 8 patients with unresectable measurable angiosarcomas showed a CR. The median survival time from the start of the treatment is 23+ months for 12 patients with macro-scopic and microscopic residual disease. The progression-free survival at 6 months was 75%.
Conclusion: This trimodal treatment leads to excellent response rates at irradiated soft tissue sarcomas, it suppresses distant metastases and prolongs survival.

Methicillin Resistance In *Staphylococcus Aureus* And Coagulase-Negative Staphylococci

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Background: *Staphylococcus* species are divided into coagulase-positive staphylococci, represented by *S. aureus* and coagulase-negative staphylococci (CNS). Oxacillin has been one of the main drugs used for the treatment of staphylococcal infections; however, a large number of *S. aureus* and CNS isolates of nosocomial origin are resistant to this drug. Methicillin resistance is encoded by the *mecA* gene which is inserted in the *SCCmec* cassette.
Aims: 1) To detect the resistance to meticillin in *S. aureus* and CNS isolates from the Clinical Hospital of Botucatu Medical School, UNESP, Brazil, 2) To determine the minimum inhibitory concentration (MIC) to erythromycin, netilmycin, sulfamethoxazole-trimethoprim and vancomycin in *S. aureus* and CNS strains by the E-test technique.
Methods: A total of 150 samples were analyzed regarding methicillin resistance by detection of the *mecA* gene, agar diffusion technique using cefoxitin and oxacillin disks, screening test on Mueller-Hinton agar supplemented with oxacillin 6 µg/mL and 4% sodium chloride and E-test.
Results: A total of 102 isolates were identified as *S. aureus* and 48 as CNS. A total of 46 (45.1%) *S. aureus* and 37 CNS (81.2%) were *mecA*-positive. *S. epidermidis* was the most frequently isolated CNS species corresponding to 87.5% of all CNS strains investigated and 83.3% were *mecA*-positive. *S. aureus* and CNS also were tested in relation to erythromycin, with 56 (54.9%) and 31 (64.6%) isolates resistant to this drug, netilmycin, with 42 (41.2%) and 30 (62.5%) isolates resistant, trimetoprim-sulfametoxazol with 46 (45.1%) and 35 (72.9%) isolates resistant, respectively. All the isolates of *S.aureus* were *sensitive to vancomycin* and 12(25%) of CNS were *vancomycin-intermediate*.
Conclusions: 1) Most of the strains of *S. aureus* and CNS was *mecA*-positive. 2) *S. epidermidis* was the specie with a higher percentage of resistance to methicillin. 3) Among phenotypic methods, E-test yielded the best results in the detection of methicillin resistance. 4) The distribution of resistance to drugs showed difference between methicillin-*resistant* Staphylococcus and methicillin-*sensible* isolates, with higher percentages of resistance to erythromycin, netilmycin and trimetoprim-sulfametoxazol in methicillin-resistant strains.
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Coagulase-Negative Staphylococci Oxacillin Resistant And Toxigenic Isolated In Brazil

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Background: Oxacillin resistance in isolates of coagulase-negative staphylococci (CNS) have emerged over the last years in neonatal intensive care units. Aims: 1) To identify CNS species. 2) To detect the resistance to meticillin in CNS strains. 3) To detect gene and mRNA expression of toxins by RT-PCR in clinical samples obtained from newborns hospitalized at the Neonatal Unit, University Hospital, Botucatu Medical School, Brazil.
Methods: In the present study, 90 CNS strains isolated from different clinical materials of newborns were investigated for the testing of susceptibility to oxacillin and by PCR for the presence of genes encoding staphylococcal toxins A (*sea*), B (*seb*), C (*sec-1*) and D (*sed*) and TSST-1 (*tst*). Strains positive for the presence of one or more genes were tested by RT-PCR for the expression of mRNA encoding the respective toxins.
Results: *S. epidermidis* was the most frequently isolated organism, corresponding to 71.1% of all CNS strains investigated and 63.5% showed oxacillin resistance. The results showed a total of 49 (45.4%) CNS strains that were positive by PCR and 65.3% were oxacillin resistant. Analysis of mRNA expression by RT-PCR detected six (14.0%) CNS strains producing SEA and SEC, with 83.3% these isolates presenting resistance to oxacillin.
Conclusions: 1) *S. epidermidis* was the most frequently isolated CNS species. 2) All strains identified as CNS by the phenotypic technique were confirmed by the genotypic method. 3) The toxin genes *sea*, *seb*, *sec-1* and/or *tst* were detected alone or in combination in all CNS species isolated, except for *S. simulans*, with the most found to be oxacillin resistant. 4) Analysis of the production of staphylococcal toxins in CNS by RT-PCR confirmed the toxigenic capacity of *S. epidermidis* and *S. lugdunensis*. 5) Most of the *S. epidermidis* that was positive for expression of mRNA that encode staphylococcal toxin were found to be oxacillin resistant.
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Pharmacokinetics (PK) And Pharmacodynamics (PD) Of Fosfomycin (FOF) For Central Nervous System (CNS) Infections

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Background: The emergence of infections caused by multiresistant bacteria call for "new" therapies. FOF could be used as part of a combined therapy for CNS infections since it shows favourable PK properties such as low molecular mass, negligible protein binding and good penetration into the uninflamed and inflamed cerebrospinal fluid (CSF). On the other hand, PD data of FOF in the CNS are scarce.
Methods: We conducted a selective literature research to compile *in vivo* PK/PD data of FOF in CNS infections.
Results: Few reports have described PD data of FOF in CNS infections in humans and in animal models. FOF peak CSF levels, half life ($t_{½}$) and penetration into CSF are shown in the table. In the rabbit meningitis model, a bactericidal effect on a penicillin-susceptible and a cephalosporin-resistant *Streptococccus pneumoniae* was only reached when FOF levels in the CSF were 8-10 times higher than the MIC value of tested strains (Nau R, *et al*, J. Antimicrob. Chemother. 1995; Ribes S, *et al*, J. Antimicrob. Chemother. 2006).

	Dose	Peak CSF levels (□g/ml)	$t_{½}$ CSF (h)/ $t_{½}$ serum (h)	% Penetration AUC_{CSF}/ AUC_{serum}	Reference
Human	5 g/8h	45.5	24.03/3.43	45.21*	Friedrich H, *et al*, Immun.Infekt.1987
	5g single dose	10. 1± 1.5	ND/2	9.3	Pfeifer G, *et al*, J. Clin. Pharmacol. Res. 1985
	8g single dose	43 ± 20	ND/3.0	23	Pfausler B, *et al*, J. Antimicrob. Chemother. 2004
	8g/8h for 3d	62 ± 38	ND/4.0	27	
Rabbit	300 mg/kg/6h	58.40 ± 21.81	5.01/3.09	49.2	Ribes S, *et al*, J. Antimicrob. Chemother. 2006

* calculated from reported data

Conclusions: The MIC of the causative strain is of paramount importance for the PD of FOF in CNS infections since the achievement of appropriate CSF drug concentrations are associated to clinical outcome.

Asparagine Synthetase Inhibitors With Nanomolar Potency: An Unexplored Approach To Treating Drug-Resistant Leukemia

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Background: Clinical studies have identified an intriguing inverse correlation between the levels of asparagine in the blood and the susceptibility of leukemia cells to chemotherapy. Leukemia cells seem to become drug-resistant by over-expressing asparagine synthetase (ASNS), an enzyme that catalyzes the intracellular synthesis of asparagine. Compounds that inhibit human ASNS represent potential drugs for treating acute lymphoblastic leukemia (ALL).
Aims: 1) To examine the effects of a potent ASNS inhibitor on the growth of a MOLT-4 cell line. 2) To develop an atomistic understanding of interactions formed between the inhibitor and ASNS.
Methods: The ASNS inhibitor was incubated at 0.1-1.0 M concentration with asparaginase-resistant MOLT-4 cells, which exhibit unregulated levels of ASNS expression. Cell viability was determined after 48 h using a WST-1 proliferation assay. The mean cell titer of treated samples was calculated, as the mean ± SD of triplicate experiments, relative to control cells. A model of the ASNS/inhibitor complex was built by homology to the structure of *Escherichia coli* AS-B, with the inhibitor being placed in the synthetase active site using flexible docking and molecular dynamics methods. Site-specific mutants of Glu-348 in *Escherichia coli* AS-B were expressed and purified by standard methods, and assayed using both steady-state kinetics and NMR-based ^{18}O-transfer experiments.
Results: In the presence of L-asparaginase, the ASNS inhibitor suppressed the proliferation of an asparaginase-resistant MOLT-4 cell line in a dose-dependent fashion. Molecular docking gave a model for the ASNS/inhibitor complex showing an interaction between a conserved glutamate side chain and a critical methyl group on the inhibitor. Assays of AS-B mutants in which this glutamate is replaced by aspartate and alanine support the assignment of Glu-348 as a general base thereby validating the computational model of the ASNS/inhibitor complex.
Conclusions: 1) This study is the first direct demonstration that ASNS inhibitors can suppress proliferation of a drug-resistant MOLT-4 cell line in a dose-dependent manner. 2) The ASNS/inhibitor model provides a firm basis for discovering novel compounds with improved activity against ALL.

Liquid Chromatography-Mass Spectrometry; A Unique Tool In Drug Development And Patient Care Via Quality Assurance, Validation Of Biomarkers And Databases, Tissue Analysis, Assessing Prognosis And Monitoring Drug Interactions

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Background: Liquid Chromatography-Mass Spectrometry (LC-MS) is a unique tool in drug development, biomarker validation, tissue analysis, assessing prognosis and monitoring drug interactions.
This is due to the accuracy and reliability of LC-MS as an analytical technique and use of more sophisticated systems capable of analysing large numbers of samples.
Methods: Initially our laboratory underwent quality assurance by analysing samples of urine and plasma for phytoestrogens (PE). Results were compared with other international institutions. As a consequence, we were certified for LC-MS analysis of PE internationally.
A database of PE content of foods was validated by comparing estimated values from the PE database with LC-MS analysis of duplicate diets[1]. Identification of biomarkers PE intake was completed by LC-MS analysis of duplicate diets and corresponding 24 hour urine collections and timed blood samples[2]. Biomarkers of PE intake were validated over time using LC-MS analysis of timed spot urine samples over a six month period[3,4]. The PE database was used to investigate the effect of PE intake on prostate cancer risk in men[5]. Both the database and biomarkers of intake were used to monitor effect of PE intake on prognosis in breast cancer patients across Scotland[6] and to monitor potential effects of dietary PE on patients' responses to cancer treatments. Uptake of PE in tumour and normal tissue was also measured[7].
Results: We were the first group in the world to produce a validated PE database and validated biomarkers of PE intake using LC-MS and employ both in prospective and retrospective studies involving cancer patients.
Conclusion: LC-MS is a unique tool for use in health care by identifying novel drugs, validating biomarkers and monitoring their actions and interactions in patients.

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Receptors For Magic Bullets. Ehrlich, Precursor Of Receptor-Mediated Drug Action. Application To Age-Related Pathologies. A Review

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Background: Medical pharmacology is based on receptor actions, using specific agonist and/or antagonist. The famous concept of Ehrlich:«corpora non agunt nisi fixata» can be considered as a founding principle of receptor theory. No drug, no magic bullet can act without beeing «recognized» by a specific molecule which mediates either its penetration in the cell of the transfer of a specific «message» to the cell interior. Without this concept no specific drug action could have been developed. Ehrlich's concept developed in the frame of his immunological experimentation, «the side-chain» concept was progresively extended to nearly all drug-cell interaction. One of the consequences of this conceptual advance was the recent increase of life expectancy, below 50years at the turn of the 19th to the 20th century to above 80 years recently.
Experimental basis: This progress was based on the application of Ehrlich's original concept to the pharmacology of age-related diseases, cardiovascular and respiratory pathologies. More recently a number of receptors were shown to decrease with age and some others «uncoupled» in aging cells from their normal intracellular transmisssion pathway with loss of its beneficial effects (Robert L, J. Gerontol, 1998,44:307-317). An example is the elastin receptor recognizing elastin sequences and triggering a Calcium transient followed by modifications of several cell functions, chemotactic movements, NO release and vasodilation but also increase of elastase and free radical release. In «old» cells the Calcium transient is dampened, Ca_i increase producing apoptotic cell death (Robert L, Labat-Robert J, Biogerontology, 2000,1:123-131). The coupling of the elastin receptor to iNOS is interrupted (Faury et al, Mech. Age. Dev.,1997,95:31-42). We could show that these alterations play a critical role in the age-dependent decline of the cardiovascular and respiratory functions (Robert et al, Biogerontology, DOI:10.1007/s10522-007-9122-6)
Conclusions: The original receptor concept of Ehrlich could be applied to aging biology and to its crucial components the mediation and modulation of the interactions of cells with macromolecules of the extracellular matrix. With this enlargement, Ehrlich's concept could be applied to aging biology and pathology and pharmacology.

Piperacillin Dosing In ICU Patients – New Magic For Old Bullets

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Background: The poor outcomes for intensive care unit (ICU) patients with sepsis require higher level research to be conducted to ensure antibiotic therapy is optimized. Altered dosing strategies may be the key to further improving patient outcomes. Aims: 1) To identify and analyze relevant pharmacokinetic studies of piperacillin in ICU patients. 2) To discuss pharmacodynamic optimization of piperacillin in ICU patients using 'non-standard' dosing strategies.
Methods: Relevant articles were identified from searches of Pubmed and the extensive files of the authors including submitted and accepted original research articles not Pubmed listed. Pharmacokinetic parameters between different patient populations were identified and compared. The effect of different dosing strategies on achieving pharmacodynamic endpoints (free piperacillin concentration maintained above the minimum inhibitory concentration of bacteria for at least 50% of dosing schedule; 50% *f* T>MIC) was evaluated.
Results: Piperacillin has been investigated in various different patient populations with differences noted between various pharmacokinetic parameters, particularly clearance and volume of distribution. Piperacillin has non-linear clearance, although this becomes effectively linear at the doses used clinically in ICU patients.. ICU patients have a higher volume of distribution and clearance than other patient populations, which results in bolus dosing producing a reduced capacity to achieve 50% *f* T>MIC. In contrast administration by continuous infusion enables superior achievement of pharmacodynamic endpoints then bolus dosing (4gm six-hourly bolus, 58%, vs continuous infusion 16gm/day, 94%).
Conclusions: 1) Various studies were identified showing altered piperacillin clearance and volume of distribution in various patient populations. 2) Dosing by extended infusion or continuous infusion achieves superior pharmacodynamic outcomes, which supports data on improved outcomes observed in previous retrospective clinical studies.

Enhancing Radiotherapy Of Cancer Using Agents That Target Thrombospondin-1 Signaling Via CD47

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Background: Radiation is a primary mode of cancer therapy that acutely damages cellular macromolecules and elicits stress responses leading to cell death. The known cytoprotective activity of nitric oxide is blocked by thrombospondin-1 (TSP1), a potent antagonist of nitric oxide/cGMP signaling, suggesting that TSP1 signaling via its receptor CD47 could correspondingly increase radiosensitivity and that antagonists of this pathway could be effective radioprotectants.
Methods: Wild type, TSP1-, TSP2-, and CD47-null mice were subjected to 25 Gy hindlimb irradiation and studied over 8 weeks. Vascular cells isolated from the respective mice were irradiated *in vitro* at 10 to 40 Gy and analyzed for survival and proliferation. Radioprotective activities of antagonists were studied in vitro in vascular cells and *in vivo* using wild type mice.
Results: Twelve hours after 25 Gy hindlimb irradiation, TSP1 null mice showed significantly less cell death in muscle and bone marrow. Two months following irradiation, skin and muscle units in the null mice showed minimal histological evidence of radiation injury and near full retention of mitochondrial function. Tissue perfusion and acute vascular responses to NO are also preserved in irradiated TSP1 null hindlimbs. The role of TSP1 in radiosensitization is specific in that TSP2 null mice were not protected. However, mice lacking the TSP1 receptor CD47 showed similar radioresistance as TSP1 null mice. TSP1- and CD47-dependent radiosensitization is cell autonomous because vascular cells isolated from the respective null mice showed dramatically increased survival and improved proliferative capacity following irradiation *in vitro*. Antisense suppression of CD47 expression effectively protected endothelial cells *in vitro* from radiation-induced death and preserved skin and muscle integrity and function in irradiated hindlimbs of wild type mice.
Conclusions: Soft tissues in TSP1 and CD47 null mice are remarkably resistant to radiation injury. This is due to a cell-autonomous radiosensitizing signal arising from TSP1 binding to its receptor CD47. Antagonists of this pathway can protect soft tissues from the deleterious effects of irradiation and thereby enable use of increased radiation doses for cancer therapy.

Present Knowledge On Pharmacogenetics Of Antiretrovirals

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Background: HIV infection is a serious but treatable disease, yet current treatment is limited by development of resistance and high rates of adverse drug reactions.
Antiretroviral therapy is especially suitable for pharmacogenetic investigation as both drug exposure and treatment response can be reliably measured.
Methods: A systematic review of the literature was carried out. PubMed's Medline was searched at http://www.ncbi.nlm.nih.gov/sites/entrez/ with the following profile: (Pharmacogenetics OR pharmacogenomics) AND (HIV OR antiretrovir*). A total of 147 articles were found. All titles were read, and a total of 19 articles were selected because of their major relevance. Abstracts of all 19 articles were studied, and among them 8 articles, according with scientific importance and recentness of publication, were finally selected. The full text of those 8 most important articles was studied in detail.
Results: Increasing knowledge about genes implicated in pharmacokinetics, mode of action, efficacy, and toxicity of drugs has already provided relevant results for clinical practice, for example:
The strong association of the abacavir hypersensitivity reaction with *HLA-B*5701* permits testing patients for the allele, and if present avoiding the drug and therefore preventing the reaction.
Persons with the allele CYP2B6*6 present higher efavirenz "area under the curve" and have increased risk of neuropsychological toxicity.
Additional gene variants are being discovered that influence the action of antiretroviral drugs. And, moreover, it is expected that larger-scale comprehensive genome approaches will profoundly improve the landscape of knowledge of HIV therapy in the future.
Conclusions: Pharmacogenetics is called to play a major role in the treatment of HIV infections in the future.

Predicting The Active Doses Of New Anticancer Agents In Humans Directly From Preclinical Data

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The success rate of clinical drug development is significantly lower in oncology (less than 5%) than in other therapeutic areas, with cost and development time of over 1 billion of dollars and more than 10 years for a drug to reach marketing approval and successful use in clinical practice. Of these drugs, few cases can really be considered Magic Bullets and, very often, their contribution proves crucial only in sub-populations of patients.
This situation clearly reflects the specific difficulties encountered in the development of oncology drugs. For example, a large number of candidates fail in the last phase of development, due to insufficient efficacy observed in the clinics. In most cases, this is not due to errors in the prediction of either the mechanism of action or pharmacological activity in humans, but to the onset of toxicity findings that prevent the achievement of the required drug exposure in patients for obtaining clinical activity. These failures in the final stage of development may have disruptive financial impact even on big multinational companies. In view of this, there is an absolute need for more appropriate ranking and selection methods of compounds in the preclinical phase.
In this talk, the improvements that can follow from a model-based pharmacokinetic-pharmacodynamic (PK/PD) approach will be illustrated. In addition, a methodology able to quantitatively predict, right from the first animal studies, the dose levels of new compounds expected to have clinical activity in patients will be presented. This estimate, combined with the toxicological, pharmacokinetic and pharmacodynamic properties of the compounds, provides a valuable input to the decision making process in drug development. On this basis, the whole development process of R&D projects may be optimised so that inappropriate compounds are discarded earlier and the real Magic Bullets are more easily recognized.

Pharmacokinetics And Effects On Adenosine Concentration Of A New 4-Anilinoquinazoline Derivative In Rats

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Background: The purinic nucleoside adenosine (ADO) regulates multiple cell functions. Its effects are mediated by at least four kinds of P1 purinergic receptors (A1, A_{2a}, A_{2b} and A3). Adenosine kinase (AK) is a key intracellular enzyme regulating intra- and extracellular ADO concentrations. Pharmacological targeting of AK can enhance ADO tissue levels. In this study, we examined the pharmacokinetics of a new adenosine kinase inhibitor, the 4-*N*-(3'-*N*,*N*-dimethylphenyl)amino-6,7-dimethoxyquinazoline (DMAQ) and its effects on ADO concentrations in rat tissues.
Methods: Male Wistar rats (n= 6 of each experimental group, body weight (BWT): 200-250g) were dosed orally with 30 and 90 mg/Kg BWT of DMAQ or vehicle (Control group – n=6). Tissue samples were collected 1h after treatment and immediately ground to a powder under liquid nitrogen. To ADO analysis it was extracted from tissues, derivatized with 2-chloroacetaldehyde and analysed by high performance liquid chromatography method (HPLC). To pharmacokinetic studies, blood was sampled in tubes containing heparin at control, 15, 30, 60, 120, 180, 240, 300, 360, 420 and 480 minutes. Samples were collected from a catheter previously implanted into the femoral artery.
Results: Oral administration of DMAQ (30 mg/Kg) was demonstrated to increase ADO concentration in rat tissues such as heart (43.0±5.5%), liver (33.4±1.8%) and brain (24.0±4.9%). Pharmacokinetic parameters were determined for orally administered DMAQ (90 mg/Kg). The DMAQ maximal concentration in plasma of 22.90 μmol L^{-1} was reached at 3h after administration and after 7h, DMAQ concentration approached the limit of HPLC quantification. DMAQ showed a rapid absorption profile with an estimated absorption half-life of 13 min. and a moderately fast rate of elimination (63 min). Values for $AUC_{0\text{-}7h}$ and volume of distribution were 970.06 μmol L^{-1} $min.^{-1}$ and 20.00 L/kg.
Conclusions: 1) DMAQ rapidly and consistently increased ADO levels in rat tissues. 2) DMAQ was shown to have favorable pharmacokinetics, indicated by a rapid absorbtion after orally dosed, wide distribution and moderate rate of excretion. Therefore, DMAQ may be considered as a good alternative for systemic inhibition of AK that can potentially be used for the treatment of cardiovascular and neurological diseases.
Authors' disclosure statement: DMAQ concentration in plasma and ADO tissue concentrations were measured by developed and validated reversed–phase high performance liquid chromatography methods.

Antimicrobial Prophylaxis In Surgery And Emergence Of Super Bugs: Dilemma In Developing Countries

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Background: Wound infections are the commonest hospital-acquired infections in surgical patients. There is a significant difference between developed and developing countries with respect to surgical site infections (SSIs). Approximately 30-50% of antibiotic use in hospitals is now for surgical prophylaxis. However, between 30-90% of this prophylaxis is inappropriate, that increases the selective pressure favoring the emergence of antimicrobial resistance. Judicious use of antibiotics in the hospital through effective antibiotic policy and guideline development is thus essential. The objective is to evaluate the reason for noncompliance in adherence to guideline for prophylactic antibiotic usage in surgery especially with developing countries in context and to recommend the practical approach for safe and efficacious use of magic bullet in this area.
Methods: We searched electronic databases including MEDLINE, EMBASE, *International Pharmaceutical Abstracts*, Surgical abstracts, Surgical Infection Prevention (SIP) database and transcripts on SSIs. Papers were retrieved and categorized according to content and recommendation status by an independent reviewer. Articles highlighting the incidence of SSIs and reason for non-adherence to guideline were extracted.
Results: Incidence of SSIs range from 7-15% in developing countries as compared to 2-5% in western set-up. Patients who develop SSIs are upto 60% more likely to spend time in an intensive care unit (ICU), 5 times more likely to be readmitted to the hospital and 2 times more likely to die than are patients without SSIs. There were numerous reasons for non-adherence to the clinical guideline. Irrational use of prophylactic antibiotic is rampant; especially the widespread use of the same to cover-up the aseptic techniques during surgery. Use of prophylactic antibiotic even for clean procedures (without recommendation) was very common. Choice of antibiotic too differs with the recommendation. Antibiotics were administered for prolonged duration (around two weeks) without supportive laboratory evidence. Emergence of Methicillin Resistant Staphylococcus Aureus (MRSA) and higher resistant organisms is very common is such setting. Major barriers involved in non-adherence to guidelines include lack of awareness about the guidelines and a general perception of guideline as a bureaucratic rather than educational tool. Local guidelines seem more likely to be accepted and followed than those developed nationally. Some practitioners perceive guidelines as "cookbook medicine" that does not permit them to make their own medical decisions. Other barriers are complex, multi-step systems that create confusion and decrease accountability. Methods for guideline adherence include surveillance and data analysis, new systems to facilitate documentation and improving workflow, education regarding current evidence-based guidelines and promoting the development of local guidelines or protocol, development and implementation of reminders to facilitate adherence to the local guidelines.
Conclusions: 1) A multifaceted educational intervention involving a team effort of healthcare professionals can have a significant effect on effective antibiotic utilization and reducing the incidence of surgical site infections 2) Local guidelines seem more likely to be accepted and followed than those developed nationally 3) Both pros and cons should be taken into consideration while administering the prophylactic antibiotic in surgery 4) Development and adherence to antibiotic guidelines is essential to prevent emergence of resistant pathogens and to rationalize the use of antibiotics in the most cost effective manner and preventing the incidence of hospital acquired infections 5) The gap between evidence-based guidelines and practice is populated by individual values, professional conflicts, and organizational conflicts that must be addressed in order to achieve optimal practice in this domain 6) Using group interviews to reveal these factors to team members and managers may be a first step to resolving the gap and reducing surgical site infections.

Systemic Immunosuppresive Therapy With Oral Sirolimus After Bare Metal Stent Implantation: The Missing Alternative To Prevent Restenosis Following Percutaneous Coronary Interventions

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Background: In previous randomized study, ORAR II (Oral Rapamycin in Argentina), we demonstrated a significant reduction of restenosis with oral rapamycin (OR) in patients undergoing bare metal stent (BMS) implantation. Its role in comparison with drug eluting stent (DES) was unknown.
Methods: Since January 2006 to September 2007 at the Cardiac facilities of three Hospitals in Buenos Aires, Argentina, 200 patients with de novo coronary lesions were randomized to treated with OR plus BMS (100 pts) or with DES (100 pts). OR was given as a bolus of 10 mg per day before PCI followed by daily doses of 3 mg plus diltiazem during 13 days after. Primary End points were to compare Hospital, Follow Up and Overall cost per patient at one, two, three and five years of follow up. Safety end point such death from any cause, Q and non Q myocardial infarction (MI) and stroke was analyzed as Major Adverse Cardiovascular Events . Target Vessel (TVR)and Target Lesion Revascularization (TLR) were independently analyzed as Efficacy end point . Direct cost in U$ dollars included procedural resources, hospitalization, medications, repeat revascularization procedures and profesional fees.
Results: Baseline demographic, clinical and angiographic characteristics were similar in both groups. At 12.1 + 6.1 months of follow up, The rates of clinically driven TLR and TVR were similar with both strategies: TVR per vessel was 8.4% with OR versus 7.7% with DES, TLR per lesion was 5.7% with OR versus 5.8% with DES (p=ns).Cumulative incidence of MACCE was not significant different, although during follow up patients treated with DES had significant high incidence of MACCE compared to OR group(13% vs 3% p=0.03) Initial and cumulative cost were significant high in DES treated patients (p=0.0001).
Conclusions: At 12 months of follow up and initial strategy with OR plus BMS is cost saving and effective compared to DES in patients with de novo lesions undergoing PCI.

Cytochrome P450 Dependent Drug Response In Oncology

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Cytochromes P450 (CYP) in families 1-3 are responsible for most of the phase I hepatic biotransformations of drugs. The polymorphic nature of these enzymes causes large inter-individual differences in drug disposition that can result in differences in drug efficacy and toxicity. In this way, specific genotypes within patients could be used to individualize drug therapy. In addition to hepatic metabolism, drug efficacy can be modified by the target cells. For example, an altered drug metabolizing capacity in cancer cells could result in resistance to anticancer drugs. Here we illustrate these different situations with two recently performed studies and present our novel findings.
Concerning the relevance of CYP variations for drug toxicities, we investigated the association between paclitaxel neurotoxicity and the polymorphisms of the CYPs mediating paclitaxel metabolism. Paclitaxel is an effective anti-cancer drug widely used, and neurotoxicity is one of its most important and often dose-limiting toxicities. The neurotoxicity has a large inter-individual variability of unknown molecular basis, and it represents a major challenge for the improvement of paclitaxel therapy. Since paclitaxel pharmacokinetic parameters have been shown to be related to neurotoxicity, we selected polymorphisms in the CYPs involved in paclitaxel metabolism, CYP2C8, CYP3A4 and CYP3A5, and investigated their association with paclitaxel neurotoxicity. In addition, we also selected polymorphisms potentially affecting paclitaxel hepatic uptake and biliar excretion, through the organic anion transporting polypeptides 1B1 and 1B3 and P-glycoprotein, respectively. In total 13 polymorphisms were genotyped in 124 cancer patients treated with paclitaxel. Three polymorphisms from the selected P450s were significantly associated with grade 2 neurotoxicity: the two alleles associated with an increased risk to develop neurotoxicity conferred a high metabolizing capacity, while the allele associated with protection was a low activity allele. On the other hand, for MDR1 the results were inconclusive and no association was found for the other genes. Altogether, the genetic variants identified in this work could be potential targets for individualized pharmacotherapy with paclitaxel, reducing neurotoxicity risks by improving paclitaxel dosing.
Concerning the efficacy of anti-cancer drugs and the genetic background of the cancer cells, we showed that the expression of CYP3A4 in peripheral T-cell lymphoma (PTCL) tumors was inversely associated to the survival of the patients. PTCLs are clinically aggressive tumors typically showing less than 30% 5-years overall survival. To date, the most effective therapy is a combination chemotherapy regimen, in many cases CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone), but the poor clinical outcome of most patients clearly reveals the need to improve the therapy by identifying factors affecting the response. Since the CYP3A subfamily enzymes are involved in the inactivation of chemotherapy drugs, we hypothesized that CYP3A expression in these lymphomas could result in a poor clinical response. We measured tumoral CYP3A and MDR1 mRNA content in 44 T-cell lymphomas finding a large variation in CYP3A expression. A high tumoral CYP3A4 expression was significantly associated with a poor drug response and survival of the patients. In conclusion, drugs efficacy and toxicity can be altered by genetic variations in CYPs, in the case of toxicity by altering drug hepatic disposition while, in the case of efficacy, alterations in the target cells can also be critical.

Design Of Aquasomes Loaded With Indomethacin And Their Released Profiles

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Background: Diverse technological strategies have been proposed in order to obtain nanoparticles, of a distinct nature. Kossovsky proposed a system to prepare nanoparticles transporting the so called aquasomes, whose particle size (lower than 1000 nm) is appropiate to parenteral administration.
Aquasomes have a water absorbent nature which not only makes their aqueous transport permissible but also confers the possibility of establishing non-covalent links with distinct molecules and macromolecules like proteins promoting a mayor stability compared than liposomes.
The aim of this study was to prepare nanoparticles in form of aquasomes with Indomethacin and determined their released profiles
Methods: The inorganic cores obtained from calcium phosphate were prepared from the precipitation of a 0.25 M NaH_2PO_4 solution and a 0.75 M $CaCl_2$ solution. The process variables were the level of ultrasound frequency and the influence of the sonication on the particle size of the inorganic cores. An experimental design 2^2 has involved sonication at 30 and 90 pulses of frequency after and during the precipitation of the inorganic cores.
Lactose coating. 1.0 mg of inorganic cores was resuspended into bi-distilled water and was added to a 100 ml solution of Lactose with mechanical agitation.
Drug loading. A solution 0.06 M of Indomethacin in acetone was added at a dispersion of 1mg/ml of the polyhydroxylated cores. After each step was carried out a filtration and a lyophilization. Structural analyses, particle size and morphology were evaluated by X-ray powder diffractometry, transmission electron microscopy (TEM), and scanning electron microscopy (SEM). Released profiles were determined by dialysis.
Results: The X-ray analysis of the samples and their observation through TEM and SEM allowed us to identify the inorganic calcium phosphate nucleus formation, as well as the layers of Lactose and Indomethacin. The final particle size of the aquasomes was in the range of 60 to 120 nm, with a media of 90 nm. The results of the dialysis showed that 1.5 times more of Indomethacin was released from the aquasomes than from the physical mixture at the end of 24 hours
Conclusions: The method to produce aquasomes was reproducible under the used conditions and they were able to release the drug.

Altered Morphine Metabolism In Asphyxiated Term Neonates During Prolonged, Moderate Whole Body Hypothermia Treatment

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Background: Hypothermia is a new treatment of cerebral injury caused by perinatal asphyxia. Both asphyxia and hypothermia may modify drug pharmacokinetics. We investigated whether analgesia with morphine in neonates with hypoxic ischaemic encephalopathy treated with prolonged moderate systemic hypothermia resulted in elevated serum morphine concentrations compared to normothermic infants.
Methods: Infants from one centre participating in a multicentre randomized study of moderate whole body hypothermia following perinatal asphyxia (the TOBY study), were randomly selected for treatment with hypothermia (n=10) or for standard care on normothermia (n=6). Hypothermia (33-34 0C) was started before six hours of age, and was maintained for 72 hours. All infants were treated with a continuous infusion of morphine-HCl, with the rate adjusted according to clinical status. Serum morphine concentrations were determined at 6, 12, 24, 48 and 72 hours after birth.
Results: Serum morphine concentrations at 24-72 hours after birth were, median (range), 292 (137-767) $ng.ml^{-1}$ in the infants treated with hypothermia and 206 (88-327) $ng.ml^{-1}$ in the infants on normothermia, p=0.014, despite similar morphine infusion rates, and cumulative doses. Morphine concentrations correlated with morphine infusion rate (r=0.663, p<0.0001), with cumulative dose (r=0.646, p<0.0001), and with treatment with hypothermia (r=0.441, p=0.004). Serum morphine concentrations reached a steady state after 24 hours in the normothermic infants but continued to increase throughout the assessment period in the hypothermia group. Serum morphine concentrations greater than 300 ng.ml-1 occurred more often in the hypothermia group (p<0.025), and when morphine infusion rate was >10 $\mu g.kg^{-1}.hr^{-1}$ (p<0.01).
Conclusions: Infants with hypoxic ischaemic encephalopathy have reduced morphine clearance and elevated, potentially toxic serum morphine concentrations when morphine infusion rates are based on clinical state.

Isoproterenol And Insulin: The Ying-Yang Of Regulation Of Mg2+ Homeostasis In Mammalian Cells

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Background: The investigation of the mechanisms regulating cellular Mg2+ homeostasis has highlighted a novel effect of isoproterenol and insulin on mammalian cells.
Methods: Over the years, studies were conducted in whole anesthetized animals, perfused organs, isolated cells as well as purified subcellular organelles from normal or diabetic animals.
Results: Isoproterenol administration results in the rapid extrusion of a sizable amount of cellular Mg2+ via a putative Na+/Mg2+ exchanger. This extrusion primarily mobilizes Mg2+ from cytoplasm and mitochondria. In contrast, treatment with insulin abolishes isoproterenol-induced Mg2+ extrusion and elicits per se an accumulation of Mg2+ from the extra-cellular compartment into the cell, which in certain cell types (e.g. cardiac myocytes) is associated with glucose accumulation. Further evidence of this opposite regulation is provided by diabetic conditions. The reduced or absent responsiveness to insulin, in fact, results in a marked decrease of Mg2+ within several tissues including skeletal muscles, heart, and liver, and in an enhanced responsiveness to isoproterenol or catecholamine stimulation, which further decreases tissue Mg2+ level and impairs the operation of several Mg2+-regulated enzymes.
Conclusions: These results provide a compelling evidence for the role of isoproterenol and insulin in regulating Mg2+ content within mammalian tissues. Together with results provided by other laboratories they highligth a key role of Mg2+ in regulating various cellular funcitons under physiological conditions.

Computed Tomographic Study Of The Paranasal Sinuses And Nasal Washings In Atopic Children Without Sinusitis Symptoms

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Background: the aim of this study is to correlate the extent of sinus disease with eosinophilia and inflammatory cells in nasal fluid (NF).
Methods: We studied 48 atopic children with allergic rhinitis (33 asthmatics); 13 nonatopic children served as a control group. Neither patients nor controls had sinusitis symptoms. Coronal computed tomographies (CT) were graded following a standard protocol. A sinus CT score ≥ 12 indicated extensive disease. Nasal wash cytology (NW) expressed as a number of cells/mL of volume recovered, was followed by differential counts.
Results: 9/48 patients (19%) had extensive disease, 7 were asthmatics and 2 had only rhinitis; 39/48 and 13 controls had a CT score < 12. Atopics had significant eosinophilia in NF and peripheral blood (Table). Total cell counts in NL were higher in atopics than in the control group. The differential cell counts in NW were similar in atopics regardless the CT score. There was correlation between CT score, peripheral eosinophil and NL eosinophil counts in atopics.

Group	CT score	n	Blood eosinophil	Cells/mL‡	Eosinophils	Neutrophils	Epithelial
Atopic	≥12	9	665	1305	757	137	137
	<12	39	558	578	208	86	109
Control	<12	13	148	390¥	6¥	167	113

*Mean §Nasal Fluid ‡Cell counts x 10^3/mL
¥ Mann-Whitney U Test p<0.05 in atopics (I,II) X Control (III) group
Conclusion: Extensive sinusal disease is frequent in atopics without sinusitis symptoms (19%). Cytology of NF did not identify these patients among atopics. Eosinophils may be involved in extensive sinus mucosa inflammatory changes.

New Chemical Entities For The Treatment Of Estrogen Receptor Negative Breast Cancer: *In Vitro* Mechanisms Of Action

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Background: Previously, we showed that combination therapy with natural compounds such as curcumin and epigallocatechin gallate significantly suppressed estrogen receptor (ER)-negative tumor growth *in vivo*. Therefore, we synthesized 8 novel compounds with the aim to increase efficacy and bioavailability. These compounds were screened for their ability to elicit cytotoxicity, apoptosis and alter cell cycle progression in ER-negative breast cancer cells. The most potent compounds were also examined for their oral bioavailability.
Methods: MDA-MB-231 and SkBr3 human breast cancer cells were incubated with new chemical entities (0 – 25 μM) for 5 days and cell number was determined using the sulforhodamine B assay. Cell cycle distribution was assessed using propidium iodide staining. The presence of apoptosis was assessed using Annexin-V-FLUOS/propidium iodide staining. The proportion of cells in each of G_0/G_1-, S- and G_2/M-phases as well as the proportion of apoptotic cells (as a percent of total cells) was determined using a flow cytometer. All data are expressed as the mean ± SEM from 4 independent determinations performed in triplicate. Statistical significance was determined using an ANOVA and a Student Newman-Keuls post hoc test, where $p<0.05$ was required for a statistically significant difference.
Results: Three compounds (RL75, RL84 and RL86) were less potent than curcumin. RL90, RL91 and RL92 had the highest cytotoxic potential in both MDA-MB-231 and SkBr3 cells with IC_{50}s of ~1 μM and 0.5 μM, respectively. RL91 (2 μM) induced apoptosis in 40% of cells from both cell lines. RL92 (4 μM) elicited a 2.5-fold increase in the number of cells in G_2 and a 60% decrease in G_0/G_1 phase cells. RL92 also produced a peak plasma concentration of 551 ng/ml 30 min after an oral dose of 8.5 mg/kg in female mice, while RL90 and RL91 were at the limit of detection.
Conclusions: 1) RL90, RL91 and RL92 elicited the greatest cytotoxicity in both ER-negative cell lines. 2) RL91 elicited the greatest apoptotic response. 3) RL92 elicited the greatest degree of G_0/G_1 arrest, mirroring its increase in G_2 phase cells and was orally available in female mice. 4) Currently, RL92 is our best drug candidate and is undergoing further testing in mouse models of ER-negative breast cancer.

Personalized Management Of Breast Cancer

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Basic science data provide major promise to achieve the goal of personalized medicine in clinical practice. If this goal will be achieved million of people worldwide will benefit. But multiple hurdles and challenges should be overcome and many scientists are skeptical whether this revolution in medicine is realistic or elusive.
Breast cancer managements represents one of cancer types with faster advances toward personalized prevention and treatment. Genetic *BRCA* testing allows effective both prevention and appropriate local control of healthy individual high-risk women with breast cancer family history and patients with inherited *BRCA* mutations respectively. Current standard targeted agents tamoxifen or aromatase inhibitors and trastuzumab are tailored only to patients with hormone receptor-positive and HER2-positive tumors respectively.

Here, we provide for the first time two comprehensive models for personalized prevention of BRCA mutation carriers and a surgery-guided algorithm for patients with familial or sporadic breast cancer.

Cannabis And Cannabinoids: The Forgotten Magic Bullet?

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Abstract: *Cannabis* sp. is a ubiquitous dioecious plant genus that grows wild in most countries. This plant, and its 66 pharmacologically active cannabinoids (CB), has been used for industrial purposes for at least 10,000 years and medically for more than 2,700 years. Historically, wars have focused upon the need for industrial hemp by seafaring nations. For example, Napoleons attempted to block passage of hemp from Russia to England in 1807. Because of the legal and political nature of the psychoactive compound, tetrahydrocannabinol (THC), development of CB based pharmaceutical interventions have provided the medical community with only nabilone (Cesamet®) and dronabinol (Marinol®). These synthesized CBs are used for treatment of cancer related symptoms such as nausia and anorexia. Current research has revealed that humans, and other mammals, have an intimate relationship with CBs as there is an endogenous CB system distributed throughout most organs of the body. Two endogenous CBs, anandamide and 2-arachnoylglycerol (2-AG), and exogenous CBs have been implicated in a number of disease processes including Alzheimer's disease, amyotrophic lateral sclerosis, Tourette's syndrome, diabetes, dystonia, fibromalgia, gastrointestinal disease, gliomas, hepatitis C, human immunodeficiency virus related symptoms, hypertension, incontinence, multiple sclerosis, osteoporosis, rheumatoid arthritis and sleep apnea, via the CB receptors CB_1 and CB_2. More recently, CB receptors have been shown to interact with a number of neurotransmitters, such as the opiate and glutamate systems. Generally, CBs dampen the excitatory effects of other excitatory neurotransmitters in the central nervous system; hence, the development of the novel synthetic CB for appetite control, e.g., rimonabant.
Conclusions: Once past the political hyperbole regarding the pros and cons of medical marijuana, research may indeed reveal that the CB system is one of Professor Ehrlich's "magic bullets."

Antibacterial Activity Of Glucosamine Sulfate And Chondroitine Sulfate?

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Background: Glucosamine sulfate (GS) and chondroitine sulfate (CS) were shown to delay X-Ray progression of OA. Glucosamine chloride along with CS was shown to reduce symptoms of moderate-severe painful knee OA. GS and CS supplements declined 5-year operative risk after its discontinuation. The mechanism of action of this therapy is still unknown. Recent data demonstrating the efficacy of co-trimoxazole administered as prophylaxis for urinary tract infections in relieving symptoms of patients with knee OA have raised the possibility of participation of the fecal flora in pathogenesis of OA [1]. Expression of Toll-like receptors 2 and 4 [lipopolysaccharide(LPS)-binding] has recently been found up-regulated in lesion areas of OA cartilage [2]. Innate production of tumor necrosis factor-alpha and interleukin-10 upon LPS-stimulation has been associated with radiological progression of knee osteoarthritis [3].
Objectives: To examine the antibacterial activity of GS, CS separately and both in one solution and as a trademark compound Megagluflex on E. coli growth in vitro.
Methods: Working solutions of GS-CS [Megagluflex (MGF), "American Health", NY)] in concentration ranging from 40mcg/ml to 100mg/ml and GS (Sigma), CS (Sigma) (1mg/ml, 50mg/ml) were prepared in normal saline as dissolvent. Inoculums of up to 104 /ml E. coli (strain ATCC 25922) was prepared in serum supplemented Brain heart growth media. One ml of the inoculum solution was mixed with 0.2 ml of different concentration of GS-CS, GS, CS solutions or with control normal saline and incubated in at 37°C for 16 hours. Number of colonies was counted. pH testing was performed for every mixture. We examined antibacterial properties of solutions with components of MGF: vitamine C and MnSO4 (0.166mg/ml, Riedel-De Haen) and solutions with similar pH (5.0) (HCL diluted by normal saline), osmolality (933mOsm/kg) (glucose solution with normal saline) like that of the tested MGF solution. Experiments with 3 repeatable results were taken for consideration.
Results: MGF inhibited E. coli growth significantly ($p=0.001$) in MIC of 1mg/ml and higher. Close to expired time of the drug antibacterial activity declined and persisted at concentration of 100 mg/ml. Solutions of GS and CS separately and in one solution mildly inhibited E. coli growth in one of 5 experiments only in concentration of 50mg/ml. Solutions of vitamine C and manganese sulfate, the components of Megagluflex, and control media, such as control solutions with pH and osmolality like that of MGF solution did not affect E.coli growth. MGF, GS, CS solutions were negative for bacterial contamination.
Conclusion: Our data suggest that MGF trademark compound has certain antibacterial activity against E. coli in vitro. Sustained antibacterial activity of GS, CS of another manufacturer in a separate and one solution was not found. Further trials are needed to clarify the antibacterial activity of GC.
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Genetic Polymorphisms Of Cytochromes P450 Influence The Leflunomide Treatment And Toxicity In Rheumatoid Arthritis Patients

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Background: Leflunomide is a disease-modifying antirheumatic drug that is used for the treatment of rheumatoid arthritis (RA). Upon oral absorption, it is converted to the active metabolite (A77 1726) that inhibits *de novo* synthesis of pyrimidine ribonucleotides. *In vitro* studies have demonstrated that cytochromes P450 (CYPs), mainly CYP1A2 and CYP2C19 may be involved in leflunomide activation. The hypothesis of our study was that genetic polymorphisms of *CYP1A2* and *CYP2C19* influence the A77 1726 serum concentrations and the response of leflunomide treatment in RA patients.
Methods: A genotyping approach was used to determine *CYP1A2* -163C>A, *CYP2C19**2 and *CYP2C19**17 genotypes in 112 RA patients. Trough steady-state A77 1726 serum concentrations were determined by validated HPLC with UV detection in all patients on leflunomide treatment.
Results: The leflunomide treatment was well tolerated by 62 patients, while 50 patients discontinued the treatment within the first year due to inefficacy (N= 7), toxicity (N= 37) or both (N= 6). *CYP2C19* genotypes did not inluence the treatment response (P= 0.258), while an association between *CYP1A2* C-163A polymorphism and leflunomide toxicity was observed. Patients with the *CYP1A2* -163CC genotype had a 11.9-fold higher risk for leflunomide-induced toxicity as compared to carriers of the *CYP1A2* -163A allele (P= 0.001, OR= 11.933, 95% CI= 2.793-50.980). On the other hand, mean values of A77 1726 serum concentrations were not significantly different between *CYP1A2* -163CC and *CYP1A2* -163CA+AA genotypes (43.9± 39.5mg/L and 33.7± 28.9 mg/L, respectively). Nevertheless, carriers of the *CYP2C19**2 allele had significantly lower mean values of A77 1726 serum concentrations as compared to patients with *CYP2C19**1/*1 genotype (18.4± 12.8 mg/L vs. 43.7± 33.7 mg/L, P= 0.005).
Conclusion: Our results suggest that the *CYP1A2* -163C>A polymorphism influenced the leflunomide toxicity, while genetic polymorphisms of *CYP2C19* had an impact on the A77 1726 serum concentrations in RA patients.

Dissemination And Communication: "Selling" Vaccines To Peers And The General Public

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Background: In a society that relies on herd immunity as a health protection measure, informing peers and the general public about immunisation can be precarious without access and knowledge of effective communication strategies. Aims: 1) To compare immunisation discourses on two vaccines: MMR and DTP. 2) To compare peer versus general public directed discourse. Focus include both descriptions of vaccines and the diseases they target. Special attention will be given to controversies.
Methods: Discourses analysed include: medical research articles in *the British Medical Journal* and *the Lancet*, British newspaper articles, and British websites maintained by the National Health Service or by private organisations. Discourses were coded manually. Cognitive Discourse Analysis was used and both qualitative and quantitative measures were obtained.
Results: Vaccine discourses were found to be very similar. The main differences lie in the target audience and in the purpose of the discourse. Medical research articles instigating controversies differ significantly in several respects (e.g. vague language, "name dropping" and high variability of titles for professionals) from typical ones. Patterns of "over-compensation" were found in both lay and professional discouses. The concept of herd immunity is very strikingly present even in professionals' discourse aimed at the general public, whereas peer-to-peer discourse empowers the reader in the discourse.
Conclusions: 1) Proponents, opponents and reporters on vaccines employ discourse as a key tool. 2) Different sources and different target audiences yield different discourses, with both intended and unintended drastic effects as a result.

Magic Bullets With Multiple Warheads: Multi-Targeted Antineoplastic Agents

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Background: While extensive work in the development of selective multi-targeted kinase inhibitors has been ongoing the design of multi-targeted antineoplastic agents that interact with target proteins of different functional families is much rarer. Multi-targeted antineoplastic agents are anticipated to have the efficacious advantages of drug combinations embodied in one molecule while avoiding the time, cost, pharmacokinetic and other disadvantages of developing and using drug combinations. Here we describe employment of a cross-docking approach to the design of molecules with antiproliferative activity that can potentially interact with both Bcl-XL and HDACs.
Methods: We employed structure based design taking advantage of available protein/ligand complex structural data (RCSB), and docking software (Autodock 3.0.5 and Fred). Target molecules were synthesized and characterized by NMR, MS and HPLC. Cell-line antiproliferation assay was performed for a 48 or 72 hour duration and assessed using sulforhodamine B.
Results: The initial set of 36 molecules was designed to bind and inhibit the anti-apoptosis Bcl-2 family member Bcl-XL. Molecular docking and synthetic tractability guided the design process. Molecules were successfully synthesized and characterized. Two molecules in this initial set exhibited near or greater than 50% growth inhibition in leukemia, breast, non small cell lung cancer (NSCLC) and renal cell lines at 10 μM concentration. Structure activity relationship (SAR) revealed a key hydrogen bond donor group necessary for significant activity. The active molecules were then cross-docked against other protein targets relevant to cancer. Histone deacetylase (HDAC) was identified as a potential secondary target. A second set of 6 molecules were design that replaced the key hydrogen bond donor group of the initially identified active molecules with an hydroxamic acid group. These newly designed molecules were successfully synthesized and characterized. These displayed 2 fold greater potency.
Conclusions: 1) Multi-targeted antineoplastic agents can be designed by utilizing a cross-docking approach of known active compounds against other potential protein targets. 2) Cross-docking results may be exploited by employing traditional medicinal chemistry techniques of lead optimization.

Some New Derivatives Of Vindoline, Monoindole Catharanthus Alkaloid

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Vindoline and catharanthine, monoindole alkaloids from Catharanthus roseus (L.) G. Don, are valuable precursors of oncolytic drugs, vinblastine and vincristine, used in treatment for some types of cancer. Due to the availability of Polonovsky-Potier and some kinds of oxidation reactions resulting in coupling of monomeric alkaloids into bisindole ones, the search for semi-synthetic analogues of natural precursors is still worth of interest. In this communication five new vindoline derivatives obtained by means of Suzuki-Miyaura reaction, some new (hetero)dimers and other vindoline oxidation products as well as results of their preliminary cytotoxicity tests are reported.

Cationic Liposomes:How A Magic Bullet Turns Into An Anti-Inflammatory Agent

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Background: Cationic lipids are mainly used as efficient DNA, RNA or protein carriers for gene therapy or immunization trials.Significant progress has been made in the understanding of the cellular pathways and mechanisms involved in lipoplex-mediated gene transfection but the interaction of cationic lipids with cell components and the consequences of such an interaction on cell physiology remains poorly described.
Methods: RAW 264.7 macrophage cell line was cultured in DMEM (Invitrogen) supplemented by 5%FBS, 1 mM sodium pyruvate, 1 mMglutamine and antibiotics (Invitrogen). Peripheral blood mononuclear cells (PBMC) were isolated from buffy coats (obtained from local routine blood donations) by density centrifugation.
Results: Interestingly, diC14-amidine liposomes stimulate, like LPS, myeloid dendritic cells through Toll-like receptor 4 activation . These findings suggest that cationic liposomes alone are potent immunostimulatory adjuvants that could stimulate efficiently the immune system and open the way to the use of cationic lipids as unique adjuvant components of vaccine.On the other hand, diC14-amidine liposomes inhibit CpG sequences or lipopolysaccharides- induced cytokine secretion by macrophages. This inhibitory effect was also mediated by the phospholipidic fraction of lipoproteins and synthetic phospholipids suggesting that cationic liposomes confer to these phospholipids new anti-inflammatory properties.
Conclusions: In summary, the examples reported in the present review highlight the dual nature of cationic liposomes: carriers and agents that modulate cellular responses (1). Most cationic lipids transport hydrophilic materials (DNA, RNA, proteins) into the cell but can also modify specific cellular activities(immunostimulatory and anti-inflammatory properties). Novel strategies, based on the co-existence of these two functions, have already been elaborated successfully.Combining the immunostimulatory activity of cationic liposomes with their carrier property could be a way to act simultaneously on the immune system and the target cell.
1)Lonez C, Vandenbranden M, Ruysschaert JM. Cationic liposomal lipids: From gene carriers to cell signaling. *Prog Lipid Res.* (2008), 47(5):340-7

National Surveillance of Antibiotic Resistance in Iran

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Background: Over usage and inappropriate use of antibiotics has fueled a major increase in prevalence of multi-drug resistant pathogens, leading some to speculate that we are nearing the end of antibiotic era. Based on several reports from the most important hospitals, microbial resistance to the available antibiotics become a novel complex medical problem and is one reason of death in impatiens. Event the out patients with uncomplicated infection after staying in the hospital have acquired new resistant infection with the progress to the morbidity and mortality. In this study, the pattern of bacterial resistance was evaluated in hospitals of five important states of Iran.
Methods: The samples were collected from impatients who had infections unresponsiveness to the routine classic treatments . After culturing and isolation of the bacteria, using disc diffusion and minimum inhibitory concentration (MIC), the sensitivity tests to the appropriate antibiotics for each sample was achieved.
Results: The type of bacteria were including; pneumococcus (9%), staphylococcous positive coagolase (4%) and negative coagolase (21.3%), pseudomonas aeruginosa (18.9%), klebsiella (25.6%), Ecoli (26.8%), shigella (1%), proteus (0.2%) and acintobacter (1%). Among these bacteria staphylococcus aureous, pseudomonas aeruginosa, klebsiella and acintobacters showed the most resistance to the even new appropriate antibiotics, and some species were completely resistant to the tested antibiotics, includiug cefepime and tozocine (pipeacilline plus Tazobactam).
Conclusions: This study showed a high and dangerous nasocomial microbial resistance, which needs intensive sanitary programs and hospital disinfection to control nasocomial infections. Also the clinician should determine whether antimicrobial therapy is warranted for a given patient and is one agent or combination of agents necessary to eradicate the infection with optimal dose, duration, and route of administration.

Nonmedical factor influencing the prescribing of antibacterials

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Background: Antibacterials (AB) are drugs with very specific indications. However, this indications are not always strictly followed. The reasons are numerous- from empirical treatment, inadequate or inappropriate diagnosis, absence of treatment guidelines etc. In addition, numerous non medical reasons influence prescription pattern of AB, and often significantly contribute to bacterial resistance development. This is particularly important in areas with high consumption of AB. The aim of this work was to analyse some of non medical factors influencing high use of AB in Serbia. Usage of AB in Serbia has been high for many years, with tendency to increase during the last few years (table).
Use of antibacterials (J01) in Serbia

Year	DDD/1000 inhabitants/year
1989*	24
1995*	25
2005	33.5
2007	45.7

(*Data from own studies; for 2005 and 2007 data were taken from yearly report published by National drug agency)
Results: Some of the reasons to high consumption of AB in Serbia:
1.) During the past 15 years numerous private pharmacies were open, where all drugs can be obtained without prescripition. In one municipality with private and state pharmacy during the one month total of 11350 DDD of AB were obtained, 13% of them without prescription, mostly in private pharmacy. More than 95% of the buyers did not go previously to the doctors, and bought AB as a part of self medication. More than 60% of AB belonged to doxicycline. The reason for this choice was low price and once daily administration. Ampicillin was on second place being old, well known drug. 2.) The control of prescribing of AB by the doctors is still not everyday practice. 3.) Introduction of the controll of precribing AB in outpatient practice as well as in Clinics lowered use of AB for app. 30%, without influencing the treatment of the patients, leading at the same time to significant pharmacoeconomic improvement. 4.) While the doctors do not strictly follow the guideline for the treatment of bacterial infections, simple infections are often treated with expensive AB, as well as with combinations of two or three AB leading to the effect of overtreatment and resistance developroment. 5.) Pharmaceutical industry did found open space for advertising. In situation of weak control and weak implementation of guidelines, doctors often follow industry`s advertising guidelines, what leads to unnecessary high usage of some of them. The example is usage of cefepime, whose usage increased for the last year more than twice.
Conclusion: Implementation of guidelines, educative efforts, law reforms are necessary to lower ind improve use and prescribing practice of AB.

Hepatoprotective Activity of Herbal Extracts Used in Iranian Traditional Medicine against Acetaminophen-Induced Liver Injury in Mice

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Background: Strategic location of liver between intestinal tract and the rest of the body facilitates its task of maintaining metabolic homeostasis, secretory and excretory functions in the body. Therefore, the key organ in the body is the dominant target site of specific toxins. Accordingly, liver disorders are numerous and varied. Species of Scrophularia, Plantago psyllium and Fumaria officinalis have been used in traditional medicine as a remedy for liver diseases. In the present study, effect of aqueous-methanolic extract of Plantago psyllium, Fumaria officinalis and total extracts of aerial parts of five Scrophularia species, collected from north-eastern of Iran, were investigated against acetaminophen-induced acute liver damage and mortality rate, and compared to a known hepatoprotective plant, Silybum marianum.
Methods: This study included 72 male mice (24 mice per treatment, weight: 20 ± 3 g, aerage ± SD). Tumor growth and animal survival was compared over 21 days between the following treatments: A) 20 mg/kg of drug X given every 6 h, B) 20 mg/kg of drug Y given every 6 h, or C) placebo. All doses were given as iv bolus. Unbound plasma concentrations in each mouse were determined after doses 1, 21, 41, 61, & 81 at three of the following time points: 0.25, 0.5, 1, 2, 3.5, & 6 h by ultrafiltration and validated LC-MS/MS assays. Population PK/PD models were developed in software XYZ to describe the PK, tumor growth, and animal survival. Dosage regimens were compared by Monte Carlo simulations.
Results: Acetaminophen produced 100% mortality at the dose of 1 g/kg in mice, while pre- and post-treatment of animals with Plantago psyllium and Fumaria officinalis extracts (300 & 500 mg/kg, orally twice daily for three days) reduced the mortality rate. The extracts at the employed doses, significantly lowered the acetaminophen-induced rise in the serum transaminases (SGOT and SGPT). Liver sections were also studied histopathologically to confirm the biochemical results. Pretreatment of animals with S. striata and S. variagata extracts (100 mg/kg, i.p., twice daily for 48 hrs) reduced the mortality rate and significantly lowered the rise in serum transaminases (SGOT and SGPT). Post-treatment of animals with four successive doses of S. striata and S. variagata extracts were reduced the mortality rate and significantly lowered the rise in the measured serum transaminases.
Conclusions: The results indicate that the crude extracts of Plantago psyllium, Fumaria officinalis, Scrophularia striata and Scrophularia variagata exhibited hepatoprotective action against acetaminophen-induced liver injury and reduced the mortality rate, which is comparable to that of Silybum marianum, a known hepatoprotective. These findings further validate the traditional use of plants in hepatic damage.

Under-treated painful condition may lead patients to opioid abuse

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Objective: To find the prevalence of Opioid abuse caused by a poorly treated painful disease.
Introduction (Background): Despite the physician reluctance to prescribe Opioids, the use of Opioid drugs for acute and chronic non-cancer pain are increasing. Although these drugs provide an important analgesic effect, they impose the risk of substance abuse to the addiction prone patients. The systematic literature reviews among the patients treated with Opioid drugs for acute or sub- acute painful conditions could not adequately answer whether the risk for iatrogenic addiction among these patients is relatively high. In order to get the incidence of Opioid abuse in a society in which the Opioid drugs are not easily available to treat painful conditions, this study was designed.
Design: By doing a pilot study the prevalence of Opioid- abuse following a poorly treated painful disease among the patients with the Opioid was determined and the size of the study participant was estimated. All the participants were evaluated via a psychiatric interview to determine the existence of Opioid abuse based on the criteria of Diagnostic and Statistical Manual of Mental Disorders (DSM ²V). Also Minnesota Multiphasic Personality Inventory (MMPI) test was performed for all the patients. Provided they claimed a painful condition lead them to the Opioid abuse, a thorough physical examination was performed to find the somatic signs of the nociception.
Results: Twenty-six out of two hundred fifty participants had an organic source of pain.
Conclusion: Opioids are useful drugs in the treatment of acute and chronic non-cancer painful conditions. But inappropriate indications and problems with the overuse along with the problems of under prescribing due to the fear of addiction and regular dose control could urge the patients to Opioid abuse even criminal diversion.

Vaccination in Patients with Cancer: Strategies to Prevent Influenza and Pneumococcal Disease

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BACKGROUND: Cordblood-derived (CB) is an important source of mostly unprimed stem cells. We sought to generate CB-derived dendritic cells (DC) immunotherapy against influenzavirus.
METHODS: Recombinant hemagglutinin (rHA) of H1N1 was expressed by vector pPSC12 cloned baculovirus in SF9 cells. Generation of Immature DCs. Umbilical cord blood units discarded from the MDACC CB bank was used after IRB approval. DC loading and maturation: in the presence of GM-CSF and IL-4, immature dendritic cells were loaded with rHA protein. Immature DCs were suspended at a concentration of 4 x 10^7 cells/ml, mixed with rHA and incubated for 10 minutes on ice in an electroporation cuvette. T-cell priming and re-Stimulation: matured loaded DCs were incubated at a ratio of 1:10 with autologous non-adherent PBMCs (typically 50% $CD3^+$), plus IL-12. ELISpot Assay was performed using standard method. ELISpots for putative HA peptide epitopes were performed similarly using 1x 10^5 - 2x 10^5 lymphocytes incubated overnight in 3.75 µg/ml peptide. T-cell specificity and functionality was then demonstrated by 51Cr CTL lysis.
RESULTS: rHA-specific lymphocytes demonstrate identifiable HLA-restriction. HA-primed lymphocytes (HLA DRbeta1*1503) from a different CB demonstrated a nine-fold increase in statistically large spots when restimulated with the DR15-262 epitope ($p<0.00002$). These data suggest that 1 in 1,900 of the HA-specific T-cells were DR15-262 restricted in a highly-specific fashion. Total ELISpot numbers ($p<0.0002$) and total IFN-gamma release ($p<0.00004$) between lymphocytes restimulated with DR15-262 and the control peptide were statistically distinct as well. Incubation of peptide DR15-262 in conjunction with HA-primed/DR15-lymphocytes or in conjunction with adenoviral hexon-primed (irrelevant) lymphocytes did not demonstrate significant numbers of IFN-gamma spots. ^{51}Cr CTL assay demonstrates the generation of HA-specific cytotoxic T-cell effectors. Naïve cord blood T-cells were stimulated three times with rHA-loaded dendritic cells; specific lysis of rHA-loaded autologous DC was double that of unloaded control targets at an E:T ratio of 5:1 ($p=0.05$).
CONCLUSIONS: The results demonstrate that, despite the generally naïve CB lymphocytes, influenza HA-specific responses can be generated ex vivo and could be potentially be used to enhance immune reconstitution following allogeneic stem cell transplantation.

Designing Peptidic Magic Bullets Against Bacteria

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Background: In the midst of rampant drug resistance, understanding the mechanisms of drug action can aid faster evolution of better drugs. Aim: To find roles of aromaticity, helicity and valency in the action of de novo designed antimicrobial peptides.
Methods: An amphipathic, cationic decapeptide Ac-G**X**R^+K^+**X**HK^+**X**$WA-NH_2$ was designed and peptides with **X**= didehydrophenylalanine (ΔFm, $aromatic^{helical}$) / α-aminoisobutyric acid (Um, $aliphatic^{helical}$)/ phenylalanine (Fm, $aromatic^{random}$) synthesized. Lysine branched bivalent dendrimers of all three (Ud, Fd, ΔFd) and a non-helical diastereoisomer (nhΔFd) of ΔFd were also synthesized. Minimum Inhibitory (MIC) and Bactericidal (MBC) concentrations against E.coli and S.aureus, FACS, fluorescence and electron microscopy, RPHPLC and gel electrophoretic methods were used to study mode of action.
Results: Bacteriostatic effects were nil (Um), mild and transient (Fm) and strong and persistent (ΔFm). Even though at par in binding to Lipopolysaccharide, ΔFm and Fm, but not Um, caused outer membrane permeabilization. Inner membrane permeabilization was attenuated and membrane architecture rehabilitated with ΔFm but not Fm. RPHPLC revealed that ΔFm was translocated into E.coli while Um and fragments of Fm were detected in the medium. Among monomers, only ΔFm was modestly antibiotic [MICs 110 µM (E.coli), 450 µM (S.aureus)]. However, its dendrimer ΔFd, was potent against both gram-negative E.coli (MIC 2.5 µM,MBC 5 µM) and gram-positive Methicillin resistant S.aureus (MIC 5 µM,MBC 7.5 µM). nhΔFd retained some activity against E.coli (MIC 5 µM, MBC 25 µM) but was inactive against S.aureus (MIC 55 µM, MBC 150 µM). ΔFd is a potent, non-cytotoxic, fairly stable, bacterial cell permeabilizing and penetrating antimicrobial peptide.
Conclusions: (1) Dendrimerization represents a scaffold for potentiation of antimicrobial peptides and the presence of ΔF in helical fold confers activity against both E.coli and S.aureus. (2) Potency comes from targeting both membranes and interior of cell. (2) In comparison to the subdued and sequential "membrane followed by cell interior" mode of action of the ΔFm, the strong and simultaneous "membrane along with cell interior" targeting by ΔFd potentiates and broadens its action across the gram negative-gram positive divide.

***Stenotrophomonas maltophilia* Bloodstream Infections in Haematology and Non-Haematology Patients- A 5-year survey in Southwest Wales**

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Background: Stenotrophomonas maltophilia is an aerobic, ubiquitous gram-negative bacillus which is of low pathogenicity and is rarely involved in causation of community-acquired infections. However it is increasingly becoming a hospital-acquired pathogen in patients who are immunocompromised, intensively treated or having long-term vascular lines.
Aims: 1) To carry out a retrospective study of all cases of bacteraemia caused by S. maltophilia in both haematology and other hospital patients over a 5-year period from the beginning of 2003 till 2008 in a 1500-bed tertiary referral university teaching hospital; 2) to determine 30-day mortality attributable to S. maltophilia; 3) to establish possible risk factors for S. maltophilia sepsis; 4) to determine total isolates of S. maltophilia and the susceptibility patterns.
Methods: The study involved retrieving patient data from laboratory information management systems and the National Public Health Laboratory Service's Datastore program using S. maltophilia as the key word.
Results. Over this period there were 1041 isolates of S. maltophilia from various clinical specimens from 585 patients. There were 90 episodes of bacteraemia in 69 patients during the survey period. Bacteraemias in 15 of the patients were regarded as contaminants on clinical grounds. Of the 54 patients with significant sepsis due to S.maltophilia, eighteen patients were suffering from haemato-oncological malignancies (9 had acute myeloid leukaemia, AML), while 16 were intensive care patients 4 of whom had burns, and 20 were general medical/surgical patients. The 30-day mortality rate for all patients was 46% (25/54) and in haemato-oncology patients it was 65% (11/17). The possible risk factors that were evident were immunosuppression especially in patients with AML and the use of broad-spectrum antibiotics especially the carbapenems where 63% (34/54) of all patients were recently on either meropenem or imipenem.
In haemato-oncology patients 82% (14/17) of patients were on carbapenems.
The susceptibility of S. maltophilia strains (isolates from all sites) tested by BSAC methods were as follows: amoxicillin (7%), co-amoxiclav (9%), aztreonam (26%), ciprofloxacin (11%), erythromycin (9%), gentamicin (19%), co-trimoxazole (87%), tigecycline (93%), timethoprim (3%) and piperacillin/tazobactam (88%).
Conclusions: S.maltophilia can cause significant morbidity and mortality in hospitalized patients especially those who are immunocompromised or under intensive care and the appropriate antimicrobials should be considered when it is isolated. The excessive use of carbapenems and other antibiotics may be selecting for S. maltophilia infections. Further research is urgently needed to develop antimicrobials that are active against S. maltophilia.

Polyclonal Rabbit Antiserum Against Porcine-SP-A Able to Detect Human SP-A Using Immunochemical Methods

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Background: The lung collectins SP-A and SP-D are present in a number of biological fluids and secretions what could make them disease markers. The bronchoalveolar lavage (BAL) is a sample that permits to detect the proteins secreted by the lung epithelium; therefore BAL is extensively employed as well as the serum to determine the surfactant protein concentrations. The immunochemical methods to measure the surfactant proteins in these samples are mainly some variations of ELISA, most of them, using two different monoclonal antibodies (sandwich variant). Supported by the observed cross-reactivity among surfactant proteins of different mammalian species we produce polyclonal antibodies for human SP-A immunodetection using porcine SP-A as the antigen.
Methods: New Zealand female rabbits were immunized intramuscularly with a mixture of 300 micrograms of porcine SP-A and the complete Freund adjuvant. After 8 days, a booster injection of 150 microgramas of the protein mixed with incomplete Freund adjuvant was given. The bleeding was conducted on day 0 and 30. An indirect ELISA was done using human SP-A (hSP-A) purified from patients with rheumatoid arthritis as the human antigen calibrator and h-SP-A was directly coated onto microtiter wells. The calibration curve range was 0.312 to 5.0 µg/ml using the antibody dilution 1:1,000. The curve range was based on the fact that no patient develops ARDS if at least 1.2 µg/ml of SP-A was measured in the BAL. Immunoblot analysis: Pool of human BAL samples were resolved by SDS/PAGE under reducing conditions and electrophoretically transferred to PVDF membranes before incubation with the polyclonal anti-porcine SP-A (1:500).
Results: The hSP-A detection limit using anti-porcine SP-A (1:1,000) was 0.625 µg/ml. The Western blot results showed that the antibody recognized specifically hSP-A in the pool of human BAL samples.
Conclusions: The anti-porcine SP-A is a promissory reagent to immunodetect human SP-A in the body fluids.

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A Promising Future for Cancer Immunotherapy in an Oil-Rich Country like Kuwait: An Invitation for Pharmaceutical and Non-Pharmaceutical International Collaboration

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Kuwait ranks sixth in the world in terms of oil reserve and ranks third in terms of oil exporter worldwide. The population of Kuwait reached only one million in 2008. Accordingly, Kuwait is a very wealthy country, and the average income of the Kuwaitis is one of the highest in the world.
Despite the above wealth, the incidence of cancer in the country increased by 300% in the past 10 years. Surgery, and chemo and radiation therapy are still the traditional methods used to treat the disease in Kuwait. Immunotherapy is still lacking in this country. Accordingly, and in an attempt to introduce such therapy, I have been working for the past four years on establishing the immune profiles of the neoplasm excised from the patients, as well as the immune profiles of the patients_ peripheral blood. Some interesting findings were seen during my work, and they sound appealing to start a nucleus of cancer immunotherapy in Kuwait.
Based on my findings, I have contacted the senior Kuwaiti officials enquiring about possible funding of the above nucleus. The feedback was extremely positive, and I was officially promised to be given the funding needed and more.
Therefore, I would like to use this coming prestigious EHRLICH II, 2nd World Conference on Magic Bullets as a venue to invite pharmaceutical and non-pharmaceutical international institutions to participate in establishing cancer immunotherapy in Kuwait. I believe this will add to the international efforts which have been aiming at making the dream of Paul Ehrlich come true.

Glomerular nephrotoxicity of aminoglycosides

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The body defends itself against potentially harmful compounds like drugs, toxic compounds, and their metabolites by elimination, in which the kidney plays an important role. Nephrotoxicity is a major side effect in clinical practice, frequently leading to acute renal failure. Many physiological mechanisms have been implicated in drug-induced renal injury. Aminoglycoside antibiotics are the most commonly used antibiotics worldwide in the treatment of Gram-negative bacterial infections. However, aminoglycosides induce nephrotoxicity in 10-20% of therapeutic courses Gentamicin-induced nephrotoxicity is characterized by slow rises in serum creatinine, tubular necrosis (due to their partial reabsorption by proximal tubular cells) and marked decreases in glomerular filtration rate and in the ultrafiltration coefficient (Kf). Kf regulation depends on the activity of intraglomerular mesangial cells. Tubular nephrotoxicity has been intensively reviewed previously, but glomerular toxicity has received less attention; thus, this presentation describes the glomerular nephrotoxic mechanisms of action of the aminoglycoside antibiotic gentamicin, with the aim to provide an actualized and mechanistic vision of pathways involved in glomerular toxic effects of aminoglycosides.

Vitamin utilisation pathways as antimalarial drug targets

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Background: Growth of the intraerythrocytic stage of the human malaria parasite, Plasmodium falciparum, is dependent on a constant supply of nutrients. The pathways by which the parasite utilises these nutrients are under investigation in our laboratory as viable antimalarial drug targets. The parasite has an absolute requirement for an extracellular supply of pantothenate (vitamin B_5), the precursor of the important enzyme cofactor, coenzyme A (CoA). We have previously shown that a pantothenate analogue, pantothenol, kills malaria parasites both in vitro and in vivo, but its exact mechanism of action is still unclear. Recently, it has been shown that the parasite also requires an extracellular supply of thiamine (vitamin B_1), but whether an extracellular supply of other vitamins is also required remains unclear. We have therefore set out to: (i) investigate the mechanism of action of pantothenol, (ii) establish whether an extracellular supply of riboflavin (vitamin B_2), is required by the parasite and (iii) establish whether a natural riboflavin analogue, roseoflavin, inhibits the growth of malaria parasites in vitro and in vivo.
Methods: Parasite metabolism of [^{14}C]pantothenol was monitored by HPLC. In vitro parasite growth was assessed by the [^{3}H]hypoxanthine incorporation assay. In vivo antimalarial activity was monitored using the 4-day suppression test in mice infected with P. vinckei.
Results: Pantothenol was metabolised by the parasite into phosphopantothenol and at least two additional metabolites. Parasite proliferation was significantly impaired when cultured in erythrocytes depleted of riboflavin. Roseoflavin inhibited parasite growth in vitro in the sub-μM range at physiological [riboflavin]. Increasing the extracellular [riboflavin] antagonised the antiplasmodial activity of roseoflavin, consistent with roseoflavin acting by inhibiting riboflavin utilisation by the parasite. Administration of roseoflavin to mice infected with P. vinckei reduced the parasitemia and prolonged the survival of the mice.
Conclusions: (i) Pantothenol acts as a substrate (rather than an inhibitor) of CoA biosynthesis enzymes, possibly producing non-functional metabolites. (ii) An extracellular supply of riboflavin is essential for the prolonged survival of the intraerythrocytic stage of P. falciparum. (iii) Roseoflavin inhibits the growth of malaria parasites both in vitro and in vivo.

Glucose Transporter Type 2 - Does It Pave the Way to Sporadic Alzheimer's Disease?

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Background: Sporadic type of Alzheimer's disease (sAD) is associated with brain insulin receptor (IR) signalling abnormalities. The sequence of these events in relation to the beta amyloid (Aβ) pathology can be traced only in sAD animal model, rats treated intracerebroventricularly with streptozotocin (STZ-icv). STZ enters the cell through glucose transporter type 2 (GLUT2) and selectively damages insulin producing/secreting cells and IR, as well as GLUT2. The time course of brain insulin system dysfunction following damage induced by icv application of GLUT2- and IR-toxic drug was investigated in this sAD model.
Methods: Male Wistar rats (3-4 month old, 6-8 per group) were treated bilaterally icv with STZ (1-3 mg/kg) and followed after 1, 3 and 6 months for the memory (Morris Water Maze Swimming Test), hippocampal neurochemistry (RT-PCR for insulin-1 /Ins-1/, IR and insulin degrading enzyme /IDE/, immunoblotting for IR, protein kinase B /Akt/PKB/, glycogen synthase kinase 3 /GSK-3/, IDE, tau protein, and Elisa assay for tyrosine kinase /TK/), and histology (immunohistochemistry and Congo red staining for Aβ). Data were analysed by Kruskal Wallis median and Mann Whitney U test.
Results: One month following the STZ-icv treatment IR mRNA and protein were decreased ($p<0.05$). Three months following the STZ-icv treatment Ins-1 and IR mRNA were decreased ($p<0.05$) and IR-TK activity increased ($p<0.05$). This was followed by alterations ($p<0.05$) of downstream IR signaling elements, decreased expression of Akt/PKB and p-GSK-3/GSK-3 ratio, increased expression of hyperphosphorylated tau protein, and decreased expression of IDE mRNA and protein. Aβ-like congophilic capillary aggregates (cerebral amyloid angiopathy) and $Aβ_{1-42}$ intraneuronal aggregates were found after 3 months. Decreased expression of Ins-1, IR and IDE mRNA, IR and IDE protein were found 6 months after STZicv treatment when $Aβ_{1-42}$ primitive plaques were found in hippocampal/cortical regions. Cognitive deficits were found at each time point.
Conclusions: STZ-icv induced damage of brain GLUT2 and IR leads to insulin resistant brain state eventually triggering Aβ pathology in animal sAD model.

Supported by DAAD and MZOS.

Nicotinic acetylcholine receptor containing an alpha6 subunit: target for a magic bullet?

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Neuronal nicotinic acetylcholine receptors (nAChRs) are pentameric assemblies made up of different combinations of alpha- (ligand binding) and beta- (structural) subunits. The alpha4beta2 nAChR is the most widely distributed subtype in the central nervous system. Recently it has been demonstrated that the presynaptic nAChRs in the brain dopaminergic neuron terminals consist of at least five different functional nAChR subtypes, two alpha4beta2-type nAChRs (alpha4beta2 and alpha4alpha5beta2) and three alpha6-type nAChRs (alpha6alpha4beta2beta3, alpha6beta2beta3 and alpha6beta2)(reviewed in Grady et al. 2007). The alpha6-type nAChRs have raised considerable interest because of their differing pharmacological properties compared with alpha4-type nAChRs. The distribution of alpha6 nAChRs is fairly restricted to the dopaminergic nerve terminals and the optical tract in rodent brain. One of the alpha6 nAChRs (alpha6alpha4beta2beta3) has the highest sensitivity to nicotine of any native nAChR that has been studied, to date (Salminen et al. 2004, 2007). This implies that this particular alpha6-type of nAChR is the first receptor to be activated following the initial puffs of tobacco. Functional and binding studies have yielded readily measured differences in sensitivity to nicotinic agonists and antagonists among these five nAChR subtypes. Several research groups have demonstrated that chronic nicotine treatment induces the downregulation of the alpha6 nAChRs while it is well established that other nAChRs (alpha4beta2 and alpha7) are upregulated following chronic nicotine administration. Therefore, there is a reason to suggest that while alpha6 nAChRs differ in many ways from the other neural nAChRs it could be possible to develop subtype selective compounds that would allow therapeutic manipulation of nAChRs in number of conditions such as parkinsonism or smoking cessation.

Phage Display: Opportunities for Development of Personalized Anti-Cancer Therapeutics

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A personalized therapeutics approach is particularly critical for cancer patients since they have limited time to experiment with different chemotherapies in the attempt to find the most effective and the least toxic. Additionally, cancer is a very heterogeneous disease with significant molecular differences in patients with the same tumor type and grade. Designing therapies becomes even more complex as the disease progresses due to further accumulation of mutations. Finally, cancer therapies frequently are extremely toxic to normal tissues and organs causing severe side effects rarely seen in patients with other diseases. To reduce adverse reactions and to drastically improve the outcome of cancer treatments, cytotoxic therapies have to be targeted and each patient needs to be profiled for the presence of cancer targets before the therapy is put into practice. Phage display technology that uses combinatorial libraries of peptides and proteins displayed on phage particles is an essential tool widely used for identification of cancer-specific targeting molecules. Applications for such molecules include: targeted delivery of cytotoxic agents to cancer cells, affinity isolation of specific biomarkers expressed on the cancer cell surface, profiling of cancer specimens from individual patients, and the design of peptidebased anti-cancer therapeutics. Our research group is exploring these and additional novel avenues for development of customized therapeutics for cancer patients. Here, we discuss the current status, present our recent data, and highlight the potentials of phage display in development of personalized approaches for solid tumors.

Calreticulin, a novel B-cell molecular target in gastrointestinal malignancies

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Background: Calreticulin (CRT), a calcium binding protein and cellular chaperon is suggested to play a key role in the recognition of dying tumor cells by the immune system. Calreticulin is recognized by antibodies (Ab) of some patients with autoimmune mediated hepatic and intestinal diseases. In an attempt to analyze the immune reaction to CRT during carcinogenesis, we 1) quantified serum levels of anti-CRT Ab in newly diagnosed patients with primary hepatocellular carcinoma (PHC, n=41), pancreatic tumors (PT, n=55), colorectal carcinoma (CRC, n=30) and gall bladder tumor (GBT, n=27), and patients with high risk for PHC – patients with viral hepatitis C (VHC, n=18), and for PT – patients with chronic pancreatitis (PI, n=16), 2) analyzed B-cell epitopes of CRT.
Methods: ELISA assays with human recombinant CRT or its peptides as an antigen were used for quantification of serum anti-CRT Ab. Pepscan method employing synthetic decapeptides of CRT was used for determination of immunodominant epitopes of CRT.
Results: Statistically significantly elevated levels of anti-CRT Ab were found in patients with PHC and PT (IgA, IgG, P<0.001), CRC (IgA, P<0.001) and GBT (IgG, P<0.001) when compared with healthy controls. Interestingly, statistically significantly (P<0.001) higher levels of IgA anti-CRT Ab were found in sera of patients suffering form PHC in comparison with VHC patients. Significantly higher levels of IgA Ab against CRT peptide KGEWKPRQIDNP (frequently recognized by IgA Ab of oncological patients tested in Pepscan experiments) quantified by ELISA confirmed immunodominance of this peptide for PHC and PT.
Conclusions: 1) we revealed a developed immune reaction against CRT including affinity maturation and isotype switching in patients with gastrointestinal malignancies, 2) IgA and IgG anti-CRT Ab were predominantly detected in newly diagnosed patients with PHC, while 3) patients with VHC – representing a risk group for development of PHC – produced only low levels of anti-CRT Ab.

New Approaches in the Treatment of Lymphoma Patients, R-Chop: Rapid Infusion Rituximab

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Background: Rituximab is a well tolerated treatment, but a cautious administration schedule is recommended to avoid infusion reactions. Rapid infusion over 90 minutes is feasible and safe. Since Rituximab is usually linked to other drugs with known myocardial toxicity, such as adriamycin, we decided to check the safety of a rapid infusion regimen and the presence of clinical or sub-clinical cardiac toxicity related to this method of administration.
Methods: We treated 42 patients with non-Hodgkin lymphoma with rapid infusion (over 60 minutes) Rituximab-based chemotherapy (CHOP-R), with average of 6.5 infusions of Rituximab per patient. Infusion-related events were analysed. We measured basal left ventricular ejection function (LVEF), every six months after chemotherapy, and the incidence of adverse cardiac events to assess cardiac toxicity.
Results: We had no grade 3 or 4 infusion-related events and no increased incidence or minor reactions during rapid infusions. None of the patients experienced cardiac events nor symptoms of cardiac failure during the whole observational period. Decreased in the post-treatment LVEF of over 10% was observed in 13 patients, and those with drop > 10% recovered normally. Patients with a LVEF decreased by 15% did not recover their normal level.

LVEF AFTER TREATMENT	Number of patients	(%)
Normal	20	48
Decreased (<10%)	4	10
Decreased (>10%), LVEF >50%	11	26
Decreased (>10%), LVEF <50%	2	4
Increased	5	12

Conclusions: Rapid infusion Rituximab is safe and well tolerated and when add to chemotherapy does not cause clinical cardiac toxicity although some subclinical decreased in LVEF can be observed.

Construction -Dependent DNA Liposome Efectiveness on Tuberculosis Vaccination

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Nowadays, non-viral adjuvants have been successful tested in DNA vaccination. Delivery systems such as multicomponent cationic liposomes have demonstrated transfection efficiency and elicited immune responses useful for vaccination. However, a great challenge is to create single - dose vaccines. Furthermore , the administration by a non-invasive route is comfortable and easier to reach populations in distant areas. Like ?magic bullets?, cationic liposomes composed by EPC/DOPE/DOTAP produce low citotoxicity and efficient transfection imparted by the gradual release of DNA as well as by the functionality of their components: structural (EPC) reducing citotoxicity, electrostatic (DOTAP) promoting interaction with DNA and cells and phase transition (DOPE) destabilizating the endosomal membrane for DNA delivery . In spite of that, the effects of DNA localization and partial complexation with the cationic lipid on the effectiveness of vaccination were not investigated. Using the functional EPC/DOPE/DOTAP liposomes, we produced constructions with internal and external DNA localization. In the first structure the DNA was entrapped and totally complexed with DOTAP in a sandwich like configuration. Secondly, DNA was partially complexed close to the surface of liposomes. Both contructions had the same DNA loading and charge ratio between DNA and DOTAP. The rigid control of the processing condition assured similar vesicle sizes (1-2µm) as well as reproductibility of the structures in small and pilot scale. The DNA-hsp65 was used in the liposomes due to its known successful prophylatic action against tuberculosis when administered as naked DNA. The effectiveness of vaccination was evaluated by intranasal route through CFU reduction in the lungs of immunized mice previously infected by mycobacterium tuberculosis. The results shown that when we used the external DNA-liposome construction, in a single dose with only 25 µg, a higher protection was observed with significant reduction of 1.97±0.23 log in the bacterial load compared to the saline group. No significant reduction on CFU was obtained by the internal DNA-liposome construction. This CFU reduction was the same observed when we used four doses (400 mg of total DNA) of naked hsp65 immunization by intramuscular route and also similar to BCG immunization. Besides, four doses of naked hsp65 by intranasal route did not induce reduction of bacilli number in the lungs. These results suggest an important structure ?function relationship: the effectiveness of cationic liposomes on vaccination depends on the rate of DNA delivery, which is not sustained by the structural stability only, but also it depends on the combination of DNA localization and partial complexation.

Telomerase as a Possible new Target for Cancer Treatment

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Background: Telomerase is a unique reverse transcriptase. Its main role is the protection and maintenance of telomeres, nucleoprotein structures at the end of linear chromosomes.
Most human somatic cells that do not or express low amounts of the enzyme and enter replicative senescence ones their telomeres are short and signal a DNA damage response. In contrast, tumour cells and around 85% of cancer express high levels of telomerase activity constitutively. This contributes to unlimited proliferation and cellular immortality. The broad expression of the enzyme in many cancer entities makes it an attractive target for the development of new cancer-therapeutic drugs.
Methods: We have designed hammerhead ribozymes against hTERT, the catalytic subunit of telomerase. We treated various breast and ovarian carcinoma cell lines with the ribozyme. Using isogenic systems of telomerase negative and hTERT-overexpressing fibroblast we demonstrate that telomerase protects cells from various stresses, including clinically relevant drugs such as topoisomerase I inhibitors. If telomerase is inhibited or withdrawn from tumour cells these cells have an increased sensitivity to apoptosis and DNA damaging drugs.
Results: We found that cells treated with anti-hTERT ribozymes go into fast apoptosis. Many other approaches using different agents such as anti-sense molecules, chemical inhibitors or dominant-negative mutants of hTERT rely on the effect of telomere shortening, which means that tumours with long telomeres have to go through many rounds of cell divisions until they finally end up with short telomeres and eventually go into apoptosis or senescence.
We believe that in our case, the withdrawal of telomerase functions in a telomere-length independent manner. We and other groups could show that telomerase has additional functions in addition to telomere maintenance. We could show recently that telomerase enters mitochondria upon stress and suggest that this could contribute to an increased resistance of cancer cells to apoptosis-inducing and DNA damaging chemo- or radio-therapeutic drugs.
Conclusions: Telomerase proves to be an important and exciting new target for the development of chemotherapeutic agents and anti-neoplasstic drugs.Telomerase-inhibitors, developed by other groups and companies are undergoing clinical trials at the moment and are recommended as new, highly specific anti-tumour agents.

Effectiveness of Nystatin in Polysymptomatic Patients
A randomized, double-blind study in 116 individuals selected by a 7-item questionnaire (FRDQ-7)

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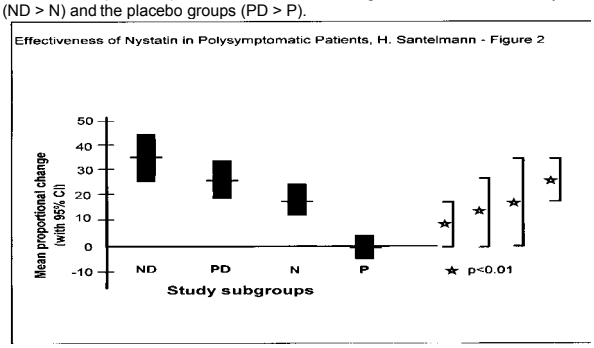
Background: Antifungal therapy has been claimed to be effective in polysymptomatic patients with diffuse symptoms from multiple body systems and even well defined diseases, traditionally not related to fungi. Hypersensitivity to fungus proteins and mycotoxins has been proposed as the cause..
Methods: We conducted a 4-week randomised, double-blind, placebo-controlled study in 116 individuals selected by a 7-item questionnaire to determine whether the antifungal agent nystatin given orally was superior to placebo. At the onset of the study, the patients were free to select either their regular diet or a sugar- and yeast-free diet, which resulted in four different subgroups: nystatin + diet (ND); placebo + diet (PD); nystatin (N); and placebo (P).
Results: Nystatin was significantly better than placebo in reduction of the overall symptom score ($P < 0.003$). In six of the 45 individually recorded symptoms, the improvement was significant ($P < 0.01$). All three active treatment groups reduced their overall symptom scores significantly ($P < 0.0001$), while the placebo regimen had no effect ($P = 0.83$). The benefit of diet was significant within both the nystatin (ND > N) and the placebo groups (PD > P).



Conclusions: Nystatin is superior to placebo in reducing localised and systemic symptoms in individuals with presumed fungus hypersensitivity as selected by the 7-item questionnaire FRDQ-7. This superiority is enhanced even further by a sugar- and yeast-free diet.

Production of cystine rich peptides and protein in E. coli: hepcidin, the iron-regulating hormone

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Background: Hepcidin is a liver produced cysteine-rich peptide hormone that acts as the central regulator of body iron metabolism. Hepcidin is synthesized under the form of a precursor, prohepcidin, which is processed to produce the biologically active mature 25 amino acid peptide. This peptide is secreted and acts by controlling the concentration of the membrane iron exporter ferroportin on intestinal enterocytes and macrophages. Hepcidin binds to ferroportin, inducing its internalization and degradation, thus regulating the export of iron from cells to plasma. The aim of this work was to develop a novel method to produce human and mouse recombinant hepcidins, and to compare their biological activity towards their natural receptor ferroportin.
Methods: Human and mouse hepcidins were produced in E coli as Thioredoxin fusion proteins. Upon cleavage peptides, were purified by HPLC and characterized. The biological activity was measured on macrophages, following the ferroportin degradation induced by hepcidin
Results: Human and mouse hepcidins, purified after cleavage from thioredoxin, were properly folded and contained the expected 4-disulfide bridges without the need of any renaturation or oxidation steps. Hepcidins were found to be biologically active, promoting ferroportin degradation in macrophages. Importantly, biologically inactive aggregated forms of hepcidin were observed depending on purification and storage conditions, but such forms were unrelated to disulfide bridge formation. Moreover this strategy was extended to the production and purification of pro-hepcidin, the 61 amino acids precursor of hepcidin. Prohepcidin was also found to contain the 4 disulfide bridges and able to generate biologically active hepcidin upon cleavage by furins (in vitro and in vivo).
Conclusions: Biologically active hepcidin and prohepcidin (its natural precursor) were produced in E coli. The thioredoxin fusion protein strategy allows correct formation of disulfide bridges in E. coli and circumvent the necessity for unfolfding/folding strategies that are necessary with other recombinant systems or synthetic peptides.

Albendazole Sulphoxide Levels in Endemic Normals and Filariasis Patients from a Lymphatic Filariasis Endemic Region of India Administered with Albendazole Using Liquid Chromatography

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Background : Determination of albendazole (ABZ) and its metabolites, albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$) in biological fluids is important to optimize dosage, length and frequency of therapy for the treatment of lymphatic filariasis. Aims: 1) To develop a simple and sensitive liquid chromatographic method for ABZSO determination. 2) To determine ABZSO in plasma of endemic normals. 3) To determine ABZSO in plasma of lymphatic filariasis patients following oral administration of 600 mg of ABZ.
Methods: A simple and sensitive reversed-phase isocratic high performance liquid chromatographic (HPLC) method for the determination of ABZ, ABZSO and $ABZSO_2$ has been developed. The mobile phase consisting of acetonitrile–water–perchloric acid (70%) (30:110:0.06 (v/v/v)) was pumped at a flow rate of 0.80 ml/min on a 5 µm, reverse phase, Discovery® RPamide C16 column with UV detection at 290 nm. The calibration graphs were linear in the range of 0.05–1 µg/ml for ABZ, ABZSO and $ABZSO_2$. The limit of quantification was 50 ng/ml for ABZ, 25 ng/ml for ABZSO and 30 ng/ml for $ABZSO_2$. The within-day and day-to-day coefficient of variation averaged 4.98 and 6.95% for ABZ, 3.83 and 6.83% for ABZSO and 3.44 and 5.51% for $ABZSO_2$, respectively. The mean extraction recoveries of ABZ, ABZSO and $ABZSO_2$ were 79.25, 93.03 and 88.78%, respectively. The method was applied to determine the plasma levels of ABZSO in 10 healthy endemic normals and 10 lymphatic filariasis patients administered with ABZ during pharmacokinetic studies.
Results: The method is suitable for the separation and determination of ABZSO and $ABZSO_2$ in a single chromatographic run. ABZSO attains peak plasma concentration of 362.50 ng/ml within 2-4 hours in endemic normals while peak plasma concentrations were 884.02 ng/ml in lymphatic filariasis patients within 2 hours following an oral administration of 600 mg of ABZ.
Conclusions: The method satisfies the criteria required for an assay required for human pharmacokinetic studies. The study shows good relation between dose, plasma concentration of ABZSO and time which has therapeutic significance for the treatment of lymphatic filariasis.
Keywords: Albendazole sulphoxide, lymphatic filariasis and liquid chromatography

Evaluation of Ecotoxicological impact of xenobiotic contaminants in terms of cytochrome P450 induction in marine fishes

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The use of molecular biomarker in marine pollution monitoring has gained enormous importance because of the increasing trend of contamination of the coastal environment by highly persistent organic pollutants. Most of these contaminant are being biomagnified through the food chain posing a serious threat to human health on environmental carcinogensis. Among the persistent pollutants polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-dioxin (PCDDs), polychlorinated dibenzo-furans (PCDFs) are well known for their toxic potentials. The accumulation of these contaminants into the tissues of marine organisms are detoxified by the induction of cytochrome P450 enzymes. In order to assess the extent of ecotoxicological impact of xenobiotic contaminants on the marine environment the induction of cytochrome P4501A1 was studied in the edible marine fishes (Mugil cephalus, Sardinella longicep and Rastrelliger kanagurta) from the Arabian sea along the Goa coast in terms of hepatic EROD activities. The Goan coastal environment is greatly contaminated with oils because of extensive shipping activities pertaining to fishing trawler, tourist boats, cargo ships, passenger ships, iron ore carrier, burges etc. The oil found in the contaminated water, sediments and biota were composed of mostly high molecular weight polyaromatic hydrocarbons (PAHs) with wide variation (0.476–5.882ug/L in water, 1.342–5.104ug/g in sediments and 8.54–37.89ug/g in biota) along the coast of Goa. The variation of EROD activities in fishes were in the range of 0.4335–3.377 pmol/mg/min along the Goa coast clearly indicating the extent of contamination of the coastal water by xenobiotic compounds such as PAHs, PCBs etc. Apart from the oil contamination, the coastal water also received huge amount of industrial wastes containing various types of xenobiotic compounds from the peripheral industries. The enhanced hepatic EROD activities in edible marine fishes clearly provides early warning signal of environmental carcinogenesis as evident from the production of reactive oxygen species via cytochrome P450 enzyme induction leads to the formation of DNA adduct resulting into DNA strand breaks.

Chitosan-stealth polymeric nanoparticles for mucosal insulin delivery

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The gastrointestinal uptake of proteins like insulin can be improved by association to nanoparticles, protecting insulin from degradation in the gastrointestinal tract, providing excellent penetration into the intestinal mucus layer and prolonging the retention of insulin in the absorption window. Further, nanoparticles are able to be taken up by the M cells of the Peyer's patches, a type of lymphatic island within the intestinal tract that represent the major gateway through which nanoparticles may be absorbed. Novel preparation methods to produce biodegradable chitosan-stealth polymeric nanoparticles have been developed in our group in order to improve the oral insulin bioavailability and solve major problems related to the current formulations. PLGA, solid lipids, dextran sulfate and alginate have been used as nanoparticle matrix. Alone, and coated with chitosan, insulin-loaded nanoparticles have been administered to diabetic animal model. Chitosan, the most widely employed natural polysaccharide, is able to reduce the transepithelial electrical resistance and transiently opening tight conjunction between epithelial cells and to combine with anionic sialic acid residues of the intestinal mucosa due to mucoadhesive properties. The adhesion of chitosan at the site of insulin absorption may offer various advantages for its uptake. Our experiments have demonstrated the feasibility of these different nanoparticles sizing between 250 to 750 nm to encapsulate insulin, maintain its bioactive form, provide controlled insulin release under gastrointestinal simulated conditions and markedly enhance intestinal absorption of insulin following oral administration by lowering serum glucose levels. Moreover, we have confirmed through intestinal section and Caco-2 cell monolayer permeability studies the absorption of labeled insulin and internalization of the protein, more pronounced when nanoparticles were formulated with chitosan coating, emphasize the absorptive enhancing effect of this polymer. In this talk, new and previous data are being presented and compared, highlighting the advantages and weaknesses of each system, and perspectives of optimizing formulations towards an efficient oral or other potential mucosal insulin delivery carrier.

Tumor Necrosis Factor-alpha Antibody Reduces Pain Related Behavior Induced by both Epidural Application of Nucleus Pulposus and Nerve Root Compression in Rats

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Background: Pain of lumbar disc herniation (LDH) can be induced by not only mechanical nerve root compression but also chemical factors such as cytokines involved nucleus pulposus (NP). Tumor necrosis factor alpha (TNF-alpha) plays an important role in the pathophysiology of LDH. The aim of this study is to evaluate if TNF-alpha antibody administered intravenously reduces the pain related behavior induced by application of NP or compression to the nerve root in rats.
Methods: Two experiments were conducted. Experiment 1: Left L5 partial laminectomy was performed and NP was applied to the L5 nerve root in 24 rats. The rats were divided into 4 groups. In 3 groups, anti-rat TNF-alpha antibody was intravenously administered immediately after, or 6 or 20 days after NP application. The fourth group was not treated with anti-rat TNF-alpha antibody (untreated rats). Experiment 2: Left L5 partial laminectomy was performed and stainless steel rod was inserted into the laminectomy hole to compress the L5 nerve root in 12 rats. The rats were divided into 2 groups. In one group, anti-rat TNF-alpha antibody was administered 6 days after the operation. In both Experiments, the withdrawal threshold of the plantar surface was determined 1 day before up through 28 days after the operation.
Results: Experimtent1: The withdrawal threshold of rats that had been treated with anti-rat TNF-alpha antibody immediately after or 6 days after, but not 20 days after, NP application, was significantly higher than that of the untreated rats. Experiment 2: The withdrawal threshold of rats treated with anti-rat TNF-alpha antibody was significantly higher than that of the untreated rats.
Conclusions: Anti-TNF-alpha antibody reduced allodynia induced by both NP application and compression to the nerve root. Late administration of anti-TNF-alpha antibody did not have an antiallodynic effect.

Firing the "Magic Bullets" at Brain Tumors: From Bench-to-Bedside and Back Again

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Malignant brain tumors are relatively rare but lethal cancers. The median survival of glioblastoma multiforme (GBM), the most common primary brain tumor in adults, is 12-15 months despite current state-of-the-art multimodal therapies. Recent elucidation of the molecular pathogenesis of GBM has led to a rational development of molecularly targeted agents ("magic bullets") as a novel treatment venue against this devastating cancer. Monoclonal antibodies and low molecular weight kinase inhibitors are the most common classes of molecularly targeted therapeutics. Most clinical trials of these agents as monotherapies have failed to demonstrate survival benefit in unselected GBM patient populations. Several strategies have been developed to circumvent the poor response to first-generation targeted agents in GBM. Such strategies may include inhibition of multiple targets by either multi-targeted ("magic shotgun") inhibitors or novel treatment combinations. Multi-modality combination of targeted agents with radiotherapy or chemotherapy may improve efficacy. Indeed, the most promising salvage regimen for progressive GBM at present seems to be the combination of a VEGF neutralizing monoclonal antibody, bevacizumab and chemotherapy, irinotecan. This regimen is associated with remarkable radiographic response rate and significant survival benefit in patients with recurrent GBM. Recently, we have identified tumor molecular profiles that may predict radiographic response and survival benefit in patients treated with this combination regimen. High tumor VEGF expression was associated with increased likelihood of radiographic response, while tumor hypoxia as measured by high expression of carbonic anhydrase-IX predicts poor survival outcome. Future development of these "magic bullets" for GBM will require advances in discovery and validation of new molecular targets, improvement of therapeutic delivery and identification of biomarkers of response or resistance. Subsequently, each patient may be treated with personalized "magic bullets" based on molecular or genetic signatures.

Protective effect of plant polyphenols-containing azuki bean (Vigna angularis) on renal damage

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Background: The azuki bean has been widely cultivated and is one of the most important crops in Japan. Azuki beans contain proanthocyanidins, which are a group of polyphenols with remarkable radical scavenging activities. We investigated the effect of azuki bean seed coats (ABSC), which mainly contain proanthocyanidins and dietary fibers, on the infiltration of macrophages and the progression of renal fibrosis in two different rat models.
Methods: Expt. 1; The streptozotocin-induced diabetic Wistar male rats were divided into three groups with 0%, 0.1% and 1.0% ABSC diets. At 10 weeks, the macrophage appearance and degree of fibrosis in glomeruli were evaluated, and mRNA expression for monocyte chemoattractant protein-1 (MCP-1) were examined by quantitative RT-PCR. Expt. 2; Wistar male rats in different groups were treated with a saline (control), cisplatin (CDDP), CDDP + 0.5% ABSC diet, CDDP + 2.0% ABSC diet. At 5 weeks after the fifth CDDP injection, the macrophages appearance and the interstitial fibrotic areas were examined.
Results: Expt. 1; There was no difference in plasma glucose levels between diabetic rats treated with and without ABSC. The plasma levels of thiobarbituric acid-reactive substances in the ABSC-treated diabetic rats were significantly lower than those in the untreated diabetic rats. Histopathologically, the percentage of fibrotic areas in the glomeruli in the ABSC-treated diabetic rats was lower than in the untreated diabetic rats. Macrophages in the glomeruli and tubulointerstitium in the untreated diabetic rats showed a significant increase in number compared with the controls. In contrast, the number of macrophages in the ABSC-treated diabetic rats was smaller than that in untreated diabetic rats. MCP-1 mRNA expression increased 2.5-fold in the untreated diabetic rat kidney, while a lower level was observed in the ABSC treated diabetic rats. Expt. 2; Histopathologically, the fibrotic areas developed around the dilated or atrophic tubules in the corticomedullary junction in CDDP-treated rat kidney, whereas the extent and magnitude of the damage were reduced in the ABSC-treated rats. Macrophages in CDDP-treated rats showed a significant increase in number, compared with the control. The number of macrophages in CDDP-plus-ABSC-treated rats was significantly smaller than that in CDDP-treated rats.
Conclusions: These results suggest that ABSC suppress the increase of infiltrating macrophages in the damaged kidney, and may lead to the attenuation of the glomerular or tubulointerstitial fibrosis in these models.

Possible role for human leukocyte antigen haplotypes in hepatotoxicity and/or pancreotoxicity associated with chemotherapy

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Background: Drug-induced liver injury (DILI) and drug-induced pancreatitis (DIP) are major health problems worldwide and leading causes of acute liver failure or severe pancreatitis. In addition, DILI and DIP are the main reasons for postmarketing regulatory decisions including withdrawal. Currently, there are no reliable markers for the diagnosis of DILI or DIP. The identification of patients who are more susceptible to these unpredictable, idiosyncratic forms of adverse events is much needed. Genetic factors for susceptibility to DILI or DIP are receiving increasing attention.
Methods: Human leukocyte antigen (HLA) was analyzed by a standard microlymphocytotoxicity method in patients with DILI and/or DIP associated with chemotherapy to elucidate the immunogenetic predisposition of DILI and/or DIP.
Results: A case of terbinafine-associated fulminant hepatitis without pancreatitis showed HLA haplotype-A26, -A33, -B62, -B58, -DR9, and -DR13. A case of micafungin-associated DIP without hepatitis showed HLA haplotype-A26, -B44, -B54, -B60, -DR9, and -DR14. A case of trimethoprim-sulfamethoxazole-associated hepatitis and pancreatitis showed HLA haplotype-A2, -B24, -B56, -B62, -DR9, and -DR14. We previously shows that the exact same HLA haplotype-A33, -B44 and -DR6 is detected in a case of rofecoxib-associated pancreatitis and cholestatic hepatitis and case series of tiopronin (mercaptopropionylglycine)-associated intrahepatic cholestasis. Moreover case series of ticlopidine-induced hepatotoxicity are associated with the same specific HLA haplotype-A33. All taken together, HLA haplotype-A33 may also be important for DILI associated with chemotherapy. On the other hand, HLA haplotypes-DR were common between our two cases of pancreatitis, suggesting that HLA haplotypes-DR9 and -DR14 may be linked to DIP.
Conclusions: HLA haplotype-A33 and -DR9/-DR14 may be associated with DILI and DIP, respectively. Because our presented cases are all Japanese, it is unknown whether the racial difference is involved in the pathogenesis of DILI and/or DIP besides on HLA haplotypes. Further studies regarding HLA haplotypes in patients with DILI and/or DIP regardless of race are much needed to avoid unpleasant hepatotoxicity and pancreotoxicity associated with drug therapy including chemotherapy.

Malaria vaccines for the better

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The most effective way to reduce disease an death from infectious diseases is to vaccinate susceptible populations. There is an urgent need for malaria vaccines with overwhelming scientific social and economical justification in particular in the perspective of increasing drug resistance against cheap and available drugs. However, immunity to malaria is considered hard to acquire and artificial induction of sterile protection in humans has until now only been achieved by inoculating radiation-attenuated sporozoites through >1000 infective mosquito bites. Because of the scientific and financial constraints clinical vaccine development has been slow over the past decade but has more recently accelerated. Presently there are more vaccine candidate near or in clinical development than ever. The most advanced recombinant protein vaccine will enter phase III later this year. Experimental human malaria infection (EHMI) is a powerful test for down-selection of vaccine candidates by testing efficacy under controlled conditions. We significantly improved EHMI by using RT-qPCR for parasite detection and introduction of a statistical model for parasitaemia. Moreover , we demonstrate that sterile protection can be induced markedly more efficiently by inoculation of intact sporozoites under cover of a blood-stage anti-malarial drug. We furthermore identify parasite-specific pluripotent effector memory T-cells producing IFN?, TNF? and IL-2 as promising novel immunological associates of protection. In conclusion, EHMI shows to be an excellent model for studies on immunity and protective efficacy. Our data support the development of a malaria vaccine based on whole parasites.

Intraluminal Cefotaxime – Heparin Lock' Placement in the Primary Prevention of Hemodialysis Catheter-Related bloodstream Infections among the Elderly and Diabetics

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Background: Tunnelled-cuffed catheters (TCCs) are often used in the elderly and diabetic end stage renal disease (ESRD) patients, to carryout hemodialysis (HD). Cmplications like infection and thrombosis reduce the life-span of TCCs. Aim: To investigate the efficacy of cefotaxime - heparin 'lock' in primary prevention of thrombotic and infectious complications and enhancement of TCC's survival in the elderly and diabetic ESRD patients.
Methods: Prospective, randomized double-blind clinical trial I- TCCs (n=119, placed among 113 elderly patients requiring long-term HD were randomized to either group-I having TCCs (n=59, placed in 58 patients) 'locked' with cefotaxime (10mg/mL) and heparin (5000 U/mL) or group-II with TCCs (n=60, placed in 55 patients) having catheter-restricted filling of heparin (5000 U/ ml) alone.
Prospective, randomized double-blind clinical trial II - TCCs (n=109, placed among 96 diabetic patients requiring long-term HD were randomized to either group-I having TCCs (n=51, placed in 49 patients) 'locked' with cefotaxime (10mg/mL) and heparin (5000 U/mL) or group-II with TCCs (n=58, placed in 47 patients) having catheter-restricted filling of heparin (5000 U/ ml) alone. The incidence of catheter- thrombosis, CRBSI, percent catheter survival and the patient mortality - were statistically compared using Kaplan-Meier survival analysis between the two groups in each trial.
Results: Trial I - Elderly patients with intraluminal cefotaxime / heparin lock on cumulative survival analysis test showed higher thrombosis–free TCC survival (84.7% vs. 63.3%, P=0.021), infection-free survival (68.7% vs. 31.3 %, P <0.001) and infection and thrombosis-free survival (65.0% vs. 35.0 %, P=0.006) at 365 days in group I compared with group II. Trial II- Diabetic patients with intraluminal cefotaxime / heparin lock, on survival analysis showed a superior thrombosis–free (86.3 vs. 63.8 %, P=0.023, log rank), infection-free (72.9 vs. 27.1 %, P = 0.004, log rank) and thrombosis and infection -free TCC survival (78.4 vs. 37.9 %, P=0.001, log rank) at 365 days besides having significantly lower incidence of CRBSI (3.68 vs. 1.56 episodes /1000 catheter-days, P < 0.0001) and CRBSI-related mortality (23.4 vs. 9.8 %, P= 0.015), compared with heparin-alone group.
Conclusions: Intraluminal cefotaxime-heparin 'locks' safely and effectively enhance the life-span of TCCs by lowering the incidence of thrombotic and infectious complications, among the elderly and diabetic ESRD patients.

AntiJEd: A possible therapeutic and immunomodulatory drug for Japanese encephalitis

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Background: AntiJEd, a low molecular weight dithiol, has been described as an immunomodulator and modifier of diverse biological actions in human and animal models and has also been shown to be effective in several diseased conditions. Therefore we studied the therapeutic aspect of AntiJEd in providing inhibition of Japanese encephalitis virus (JEV) infection.
Methods: Groups of mice were inoculated with JEV (10^2 LD_{50}; i.c.), followed by administration of various doses of AntiJEd (10-100 μmol/kg, i.p.) or placebo, daily or alternate day. Controls consist of mice that either received normal mouse brain suspension (= mock-inoculation) or were treated with various doses of AntiJEd (10-100 μmol/kg) alone. Mice were observed for survival for 21 days and Average Survival Time (AST) was calculated. The data were analyzed using Student's t-test and P value (two tailed) of <0.05 was considered significant.
Results: AntiJEd tested at various doses (10-100 μmol/kg) revealed that administration at low concentration (10 μmol/kg; i.p.) on alternate days prolonged the average survival time (AST) of mice infected with lethal dose of JEV (10^2 LD_{50}, i.c.) and delayed progression of the disease. The low dose also provided >80% survival in sub-clinical (10^5 LD_{50}, i.c.) JEV infection. Administration of AntiJEd to JEV-infected mice enhanced the inducible nitric oxide synthase (iNOS) activity in brain and level of serum tumor necrosis factor-α (TNF-α). We have recently demonstrated the production of nitric oxide (NO) via induction of iNOS activity is meditated by circulating macrophage-derived factor (MDF), which may be responsible for the delayed progression of the disease. AntiJEd mediated inhibition of JEV is believed to involve the augmentation of protective role of MDF as evidenced by the observation that pretreatment with anti-MDF antibody significantly decreased the AST of mice and together with the inhibition of iNOS activity. Interestingly, AntiJEd alone did not stimulate iNOS and TNF-α in mock-infected normal mice.
Conclusions: Collectively our data suggest that AntiJEd may be a possible anti-JEV therapeutic agent as it provides inhibition of JEV infection probably by inducing the iNOS activity and TNF-α and delays the progression of disease, though more work is needed to explore its role in JEV infection.

Breast and Prostate Tumor Cell Destruction by Genetically Altered Salmonella with Preferential Targeting of Mitochondria

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Background: Genetically modified Salmonella typhimurium strains present an attractive novel treatment for cancer, as Salmonella preferentially replicate within tumors and destroy cancer cells without causing septic shock that is typically associated with wild-type S. typhimurium infections. Different Salmonella strains have been used with varying results and the mechanisms by which Salmonella exploit their host cells may vary for different strains. Strain optimization is therefore important. Here we present data that show S. typhimurium strain VNP20009 and CRC1674 destroying human breast (MCF-7) and prostate (PC-3M) cancer cells by preferentially targeting mitochondria.
Methods: S. typhimurium-infected PC-3M and MCF-7 cancer cells were analyzed with fluorescence and immunofluorescence microscopy at 20min, 4hrs, 8hrs, and 24hrs after inoculation and fixed with 3.7% paraformaldehyde. Rhodamine-phalloidin was employed to stain microfilaments, FITC-anti-tubulin antibody to stain microtubules, and DAPI to stain DNA. Transmission electron microscopy (TEM) was performed at the same time points after fixation in 2.5% glutaraldehyde in 0.1M HEPES containing 0.2% tannic acid. YFP-mitochondria transfected mouse 3T3 cells were used to study the effects of Salmonella infestation on mitochondria in live cells.
Results: Our results show incorporation of VNP20009 and CRC1674 strains into Salmonella-containing vacuoles (SCVs) formed within host cells and gradual destruction of mitochondria within PC-3M and MCF-7 cells with complete loss of cristae at 24 hrs of inoculation. YFP-mitochondria transfected mouse 3T3 cells showed decreased mitochondrial fluorescence intensity after inoculation. The nucleus did not appear affected by either VNP20009 or CRC1674 strains within 24hrs.
Conclusions: Our data show that genetically modified S. typhimurium (VNP20009 and CRC1674) destroy PC-3M prostate and MCF-7 breast cancer cells with obvious destruction of mitochondria while the nucleus does not appear affected.

Depending on PKC-theta Expression, the Novel PKC Activator PEP005 can Either Increase or Decrease Apoptosis of Hemopoetic cells

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Background: Broad range activators of PKC isoforms induce apoptosis in many cancer cell lines. In T cells, however, activation of PKC can be a survival signal and a mitogen. We have been investigating the underlying mechanism explaining the difference between T cells and other hemopoetic cell lines in their response to PKC activation.
Methods: Activated $CD8^+$T cells rapidly enter apoptosis when deprived from survival factors such as IL-2 or IL-15. We cultured human resting and activated CD8+ T cells in the presence and absence of IL-2 and PEP005 and investigated the effects of PEP005 on cell-survival and proliferation. Furthermore we studied the effect of PEP005 on activation and expression of factors involved in regulation of apoptosis such as NFkB, PKCθ as well as Bcl-2 family members BIM, Bcl-xl, Bcl-2 and Mcl-1. We compared the response of CD8+ activated T cells to PEP005 to that of promyeloid cell line HL-60, which does not express PKCθ.
Results: We found that PEP005, a novel PKC activator, can replace cytokines as a survival signal. In freshly isolated naïve or central memory T cells, PEP005 inhibits apoptosis and induces proliferation. We demonstrate that the survival effect depends on activation of PKCθ. Expression of this PKC isoform is largely restricted to T cells and myocytes. Our findings suggest that incubation of T cells with PEP005 inhibits apoptosis through activation of NFkB downstream of PKCθ. It also involved the downregulation of the proapoptotic Bcl-2 family member BIM and upregulation of its anti-apoptotic counterparts Mcl-1 and Bcl-x_L.
Conclusions: We conclude that PKCθ expression determines if PKC activation increases or decreases the rate of apoptosis in haemopoetic cells.

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Rimonabant, First CB1 Receptor Antagonist, a Potential Magic Bullet to Reduce Cardiometabolic Risk in Patients with Abdominal Obesity

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Background: Abdominal obesity is associated with type 2 diabetes and dyslipidaemia, leading to an increased risk of cardiovascular disease. The endocannabinoid system is overactivated in presence of abdominal obesity, which contributes to increased cardiometabolic risk. We describe the clinical results obtained with rimonabant, the first selective cannabinoid type 1 (CB_1) receptor antagonist, in overweight and obese patients.
Methods: Rimonabant (5 and 20 mg) was first evaluated in the RIO programme: RIO-Europe, RIO-North America and RIO-Lipids in non-diabetic patients, RIO-Diabetes in patients with type 2 diabetes on monotherapy with sulfonylurea or metformin. Rimonabant 20 mg was further evaluated in type 2 diabetic patients treated with diet alone (SERENADE) or with insulin (ARPEGGIO), in abdominally obese patients (ADAGIO) and in patients with coronary atherosclerosis (STRADIVARIUS).
Results: Rimonabant 20 mg, compared to placebo, consistently induced greater reductions in body weight, waist circumference, blood pressure, triglycerides, metabolic syndrome, insulin resistance and inflammation markers, and greater increases in HDL cholesterol and adiponectin concentrations. In addition, in the diabetic population, rimonabant 20 mg was associated with a greater reduction in glycated haemoglobin (HbA1c) levels whatever the baseline therapy. Almost half of the metabolic effects of rimonabant were beyond weight loss. Favourable metabolic effects observed after 1 year persisted after 2 years. Rimonabant also reduced intra-abdominal and liver fat (ADAGIO). Rimonabant 20 mg for 18 months was associated with a non significant reduction in percent coronary atheroma volume, but a significant reduction in total atheroma volume (STRADIVARIUS). Adverse events more frequently reported with rimonabant were gastrointestinal, neurologic and psychiatric in nature, contraindicating the use of rimonabant in case of depression.
Conclusion: In both non-diabetic and diabetic overweight/obese patients, rimonabant 20 mg/day remarkably improved the cardiometabolic profile via pleiotropic central and peripheral effects. Whether CB1 receptor antagonism might be considered as a "magic bullet" capable of reducing the incidence of cardiovascular events in this high risk population is currently evaluated in the large CRESCENDO outcome prospective trial.

Sodium and Diffusion MRI as Biomarkers of Initial Tumor Response to Therapy in Rodents

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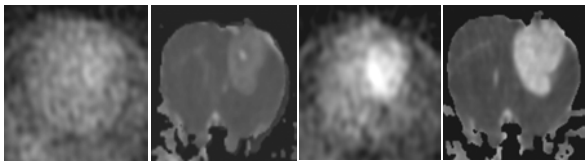
Background: The finding that, during therapy, tumors' sodium content correlates with corresponding alterations of water diffusion is attracting keen attention in the efforts to understand and further develop surrogate MRI biomarkers for tumor therapy. It is especially noteworthy that changes in both sodium and diffusion take place in the first days after therapy. Both types of imaging are able to detect a heterogeneity of tumor response and have potential to predict future individual tumor responses.
Methods: Rat 9L gliosarcoma cells were implanted intra-cranially or subcutaneously in male Fisher 344 rats (weight ~ 120 g). In ~14 days after tumor implantation, animals were subjected to a single ip BCNU chemotherapy with two different doses (13 & 26 mg/kg). Each 2-3 days, tumor growth, together with 3D sodium MRI and diffusion map were detected following the treatments. The previous experiments were performed using 9.4T MRI scanner and the latest results were acquired using 21T magnet.
Results: The overview of sodium/diffusion MRI studies of rodent glioma and 9L subcutaneous tumors will be discussed, including the latest data acquired using the ultra high magnetic field of 21.1T available at NHMFL. Representative MR images of sodium and diffusion map acquired before and seven days after BCNU therapy (26 mg/kg) demonstrate dramatic increases in tumor sodium and ADC. At 21T, a unique sodium resolution of 1μL was attained (Fig. 1).



A B C D

Fig. 1. *In vivo* sodium and diffusion map MRI of rat glioma before (A, B) and 7 days after BCNU chemotherapy (C, D), respectively, for the same animal and slice position.

Conclusions: Sodium MRI and proton diffusion exhibit early and strong correlated responses in rodent models during tumor treatments equally in brain and subcutaneous tumors. Both methods revealed a decreased response if therapy doses were decreased or if tumor acquired an additional resistance. The ultra high field experiments at 21T allowed a record high resolution for sodium and demonstrate a unique sensitivity of sodium MRI to tumor therapy. These two imaging modalities can be valuable biomarkers for individual evaluation of in vivo therapeutic cellular changes and for developing new drugs for tumor therapy.

Targeting of nitric oxide-donors to the phagosomal compartment of macrophages and the release of NO mediate killing of intracellular Leishmania parasites within the host cell

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Leishmania are intracellular protozoan parasites. After their transmission to mammalian organisms they mainly reside in the phagosomes of macrophages, which either serve as host cells or - after activation - as antileishmanial effector cells. Efficient control of Leishmania in these cells requires the enzyme inducible nitric oxide synthase (iNOS), which is expressed following activation of infected macrophages by interferon (IFN)-gamma and/or tumor necrosis factor (TNF) and metabolizes L-arginine into citrulline and nitric oxide (NO). NO is known as reactive radical with potential toxic effects.
Here, we present data that clearly support the idea that NO itself or reactive substances generated by NO represents the metabolite responsible for the killing of the parasites. First, the addition of a NO-scavenger to Leishmania (L.) major-infected macrophages stimulated by interferon (IFN)-gamma and TNF led to a significant increase of parasite proliferation. Second, transwell and co-culture experiments with infected iNOS knockout (ko) macrophages and IFN-gamma/LPS-stimulated wildtype (wt) macrophages revealed that the destruction of Leishmania in the iNOS ko cells was dependent on the close proximity of iNOS wt macrophages, indicating that unstable and reactive NO mediated the kill. And third, we were able to generate NO-donors targeted to the phagosomal compartment by endocytosis that finally induce killing of the intracellular L. major parasites within the vacuoles without toxic effects on the host cells. These prodrugs of NO are characterized as O^2-glycosylated diazeniumdiolates consisting of mono- or disaccharides (e.g. N-acetylglucosamine) that are recognized and transported by the mannose receptor (MR) of macrophages and glycosylated diazeniumdiolate ions that, in their free form, spontaneously release molecular NO at physiological pH. Depending on the attached sugar the O^2-glycosylated diazeniumdiolates were more or less stable under physiologic conditions (pH7.4), but macrophages were shown to be capable of metabolizing the compounds resulting in the release of NO. This macrophage-induced accelerated hydrolysis of the glycosides was sufficient to exert potent antiparasitic effects indicating the potential utility of O^2-glycosylated diazeniumdiolates for delivering therapeutic levels of NO to intracellular pathogens resident in infected macrophages.
Together, these data led us to conclude that iNOS-dependent killing of intracellular Leishmania parasites depends on direct toxic effects of NO and pharmacological supplementation of the macrophages' NO reserves might restore the immune system's capacity to contain and eliminate these pathogens.

A Role for Macroautophagy in Antiestrogen Resistance

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Background: Adjuvant antiestrogen therapy of estrogen receptor positive breast cancer is effective for approximately 70% of patients, while 30% of patients either do not respond or develop resistance to the antiestrogen being administered. Typically, antiestrogen treatment of breast cancer cells leads to a cytostatic response, with only a small percentage of cells actually dying due to the cytotoxic action(s) of the antiestrogen. We hypothesized that breast cancer cells surviving antiestrogen therapy undergo the catabolic process of macroautophagy and that macroautophagy facilitates the development of antiestrogen resistance.
Methods: To test our hypothesis we have utilized several breast cancer models. Molecular, biochemical, histological and imaging methods were used to identify the induction of macroautophagy and the autophagosome-the functional organelle of macroautophagy, breast cancer cell proliferation, and death in response to hormonal therapy. Autophagosome function was monitored by analyzing the levels of two proteins key to the function of the autophagosome- the lipidated form of light chain 3, designated LC3 II, and p62. In addition, long-lived protein turnover was assayed as a measure of autophagosome function.
Results: We have determined that antiestrogen therapy induces macroautophagy and that blockade of autophagosome function can convert the cytostatic action of antiestrogen treatment to a cytotoxic (death promoting) action. Small molecule inhibitors of autophagosome function as well as siRNA knockdown of macroautophagy-inducing genes were shown to induce apoptosis in breast cancer cells. Importantly, we were able to demonstrate that macroautophagy facilitates the development of antiestrogen resistance by utilizing a step wise drug selection protocol in which breast cancer cells were exposed to incremental doses of 4-hydroxytamoxifen.
Conclusions: Macroautophagy is now being appreciated as a major survival mechanism in cancer cells under a variety of stress conditions. Our studies now uniquely demonstrate that macroautophagy facilitates the development of antiestrogen resistance. Our current studies are aimed at identifying small molecule inhibitors of autophagosome function, such as chloroquine, that may have potential use as an adjuvant to antiestrogen therapy in clinical trials.

High Throughput Screening for In Vitro Toxicity Screening: A Gradual Acceptance of New Test Methods

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Background: Early in vitro toxicity screening within the pharmaceutical industry has several advantages. A large number of compounds can be tested with small amounts of compound at a low cost price. Moreover deselection can take place at an early stage. The focus in this review is on genotoxicity, cytotoxicity, nuclear receptor activation as well as phase I and II enzyme competition assays.
Results: For genotoxicity, enzyme mutation and SOS repair are studied with Salmonella in Ames II (Xenometrix) or Vitotox™ (Thermo) assays. Chromosomal aberrations are studied with a RAD54 promoter activation in yeast with Green Fluorescence Protein (GFP) Greenscreen (Gentronix) or ß-galactosidase Radarscreen (ReMynd) as read-outs. The Vitotox™ assay is a predictive substitute for the full Ames test, while the RadarScreen assay is one for chromosomal aberration and micronuclei tests. For cytotoxicity, human liver HepG2 cells are used to determine glutathione depletion (Monochlorobimane), calcein uptake (Calcein-AM), mitochondrial failure (Alamar Blue, O_2 consumption Luxcell), DNA proliferation (Hoechst 33342), and radical oxygen species activation (Dichlorofluorescein) or NRF2 Responsive Element mediated luciferase activation. These assays predict cytotoxicity towards a confidence level of 75%. Nuclear activation can be induced for the Arylhydrocarbon Receptor (AhR), Pregnane X receptor (PXR), Constitutive Androstane Receptor (CAR), Retinoic Acid Receptor α and ß (RARα and ß), Thyroid Receptor α (TRα), Liver X Receptor α (LXRα) or Farnesoid X receptor (FXR) in human liver HepG2 and/or H4IIE cells. This can be measured by Q-PCR or by the use of specific substrates for the individual cytochrome P450 enzymes. Competition assays for Cytochrome P450 and UDP-glucuronosyltransferase are available and show good predictivity for human enzymes.
Conclusions: The strategy is to implement these assays in the early phase of toxicity testing. This means that all these the assays are performed for a ranking ot the lead optimisation compounds. However, due to the high demands on purity of the compound for genotoxicity testing and the overall amount of compound needed for all these assays, i.e. 10 to 20 mg, in principle only after the first positive identification of the pharmaceutical activity in vivo and in vitro.

Non-steroidal Anti-inflammatory Drugs; a Potential New Way to Treat Human Cytomegalovirus (HCMV) Disease

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Background: HCMV is responsible for a great amount of personal suffering as well as for significant economical damage. As of today there are three systemic drugs approved for HCMV treatment: ganciclovir, foscarnet, and cidofovir. These drugs have provided major advances in HCMV disease management, although they are limited by toxicities, oral bioavailability and efficacy. Furthermore there are reports of cross-resistance for the major drugs. Therefore new drugs, preferably in oral formulations, are needed. Of particular need are drugs which could be used in congenital HCMV disease in neonates.
Methods: Experiments have been conducted in primary human foreskin fibroblasts with the HCMV strain AD169. Cells were pre-treated with medium containing the indicated amount of tolfenamic acid for 24 h before inoculation with the indicated amount of virus. The inoculum was replaced by fresh drug-containing medium, which was replaced with fresh drug-containing medium every 24 h. Data were collected via fluorescenct focus assay, immunofluorescence, quantitative PCR & quantitative RT-PCR.
Results: Human cytomegalovirus has previously been shown to induce the accumulation of cyclooxygenase-2 RNA, protein and enzyme activity. High doses of cyclooxygenase enzyme inhibitors substantially block viral replication in cultured fibroblasts. Here we demonstrate that two nonsteroidal anti-inflammatory drugs, tolfenamic acid and indomethacin, reduce direct cell-to-cell spread of HCMV in cultured fibroblasts by about 50 %. The block occurs at concentrations of drug commonly used for human therapy (~ 20 µM), and it is reversed by addition of prostaglandin E2 (PGE2), proving that it results from the action of the drugs on cyclooxygenase activity. It is noteworthy that Tolfenamic acid is well bioavailable and has usually mild side effects even in higher doses over a prolonged period of time and there are trials in pediatric patients.
Conclusions: Our study has been designed to ascertain the therapeutic potential of cyclooxygenase inhibitors as an alternative antiviral treatment. Since direct cell-to-cell spread likely contributes importantly to HCMV pathogenesis, we suggest that non-steroidal anti-inflammatory drugs might help to control HCMV infections, either as a monotherapy or in conjunction with other anti-viral treatments.

PK/PD Modeling of Time-Kill Curves: Addressing Biphasic Killing Patterns

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Background: Bacterial time-kill curves can be normally modeled with simple modified indirect response E_{max} models. These models have been successfully applied to describe effects of β-lactams (penicillins, cephalosporins, and penem drugs), against various strains of bacteria. However, quinolones have been shown to exhibit a biphasic killing pattern, which cannot be described by the models applied to β-Lactams. In this work we evaluated different Pharmacokinetic/Pharmacodynamic (PK/PD) models to describe the biphasic killing pattern exhibited by ciprofloxacin (CIP), a fluoroquinolone, in in vitro time-kill experiments.
Methods: E. coli, P. aeruginosa, and S. aureus were exposed to changing concentrations of CIP in an in vitro model of infection, in which the 4 hour in vivo half-life of CIP was simulated. Static concentration kill curves for CIP were also constructed againt E. coli and S. pneumoniae. In all experiments, samples were collected at predetermined time points. Three 10 to 100-fold serial dilutions were plated in duplicates and incubated overnight at 37º C. Time-kill curves were obtained by plotting the number of CFU/mL against time. Different PK/PD models were applied to describe the in vitro time-kill kinetics of CIP in these experiments.
Results: Biphasic killing pattern was observed against all three bacteria in the in vitro model of infection, as well as for E. coli in the static concentration time-kill experiment. The biphasic killing pattern against these strains was successfully described by both a novel Adaptive E_{max} model, which included a term to account for the change in the kill rate after approximately 4 hours, as well as a two sub-population E_{max} model. EC50 values of 0.0035 mg/L for E. coli, 0.0129 mg/L for P. aeruginosa, and 0.078 mg/L for S. aureus were obtained. The model allowed good individual fits for multiple-dose data extracted from the literature. However, kill-curves against S. penumoniae did not present the biphasic pattern, and were successfully modeled by the same modified indirect response E_{max} models applied to β-lactams.
Conclusions: 1) Biphasic killing kinetics was confirmed for CIP against 4 different strains of bacteria, but not against S. pneomoniae; 2) The biphasic killing pattern was described successfully by 2 very different models; 3) These results do not provide insight into the mechanism of this observation.

Bringing Light Into the Dark: Influence of Fluorescence Labeling on Protein Nanoparticles for *in-vivo* Use

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Background: Protein nanoparticles (NP) are one of the most promising tools for drug delivery in the field of tumor therapy. While scientific research advances from basic *in-vitro* tests towards complex *in-vivo* studies, the visualization of those NP within various animal systems becomes of increased interest. Several groups have used fluorescent dyes or quantum dots with the drawbacks of unintendedly changing the NP properties and very low signal intensities *in-vivo*. We investigated several labeling techniques and dyes for their influence on size, aggregation and surface charge.
Methods: NP were prepared from gelatin type A by two-step desolvation. Different commercially available fluorescent labels were incubated with the protein solution prior to NP formation or with unmodified NP. All covalent labeling steps were done according to the manufacturer's protocol, while dextran dyes were incorporated into the gelatin matrix. Cationisation was done with cholamine hydrochloride or with diethylaminoethanol-dextran. The read out of fluorescence intensity of the standards and NP was conducted with a fluorimeter and a fluorescence microscope. Size and zeta were analysed with dynamic light scattering prior and after each modification step.
Results: The signal intensity was comparable to the extinction coefficient from the dye manufacturers. The signal was factor 11.2 (SD 3.4) to 83.2 (SD 10.2) lower depending on the dye. NP in full blood showed even weaker fluorescent signals that could be increased by max. 24.5% with cell lysis. In a range of 0.02 mg/ml to 1 mg/ml dye no significant changes in NP size and surface charge were observed if neutral dyes were used. pH sensitive dyes lead to an increase in NP size and a drop in zeta potential of the cationic NP to the level of unmodified NP. Matrix incorporation resulted in a reduction of the zeta potential but in a growth from 189 nm (SD 2.9) to 201 nm (SD 4.2). Statistical analysis of the data was performed by a one way analysis of variance.
Conclusions: 1) NP were labeled successfully with strong fluorescent dyes without major changes in the inherent properties at low concentrations and if applied prior to NP formation. 2) NP characteristics are influenced by the charge, steric properties and pk_a values of the used fluorophores. 3) Finally the signal intensity of our NP was maximized towards fast and reliable *in-vivo* detection.

Transferable Resistance to Five Different Classes of Protein Biosynthesis Inhibitors Including Oxazolidinones Mediated by the Gene cfr in Staphylococci

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Background: Analysis of staphylococci with elevated MICs of florfenicol (32 - ≥128 mg/L) identified two novel resistance genes, fexA and cfr. While the gene fexA codes for a phenicol specific exporter, the mechanism of cfr-mediated resistance was unknown. Since enzymatic inactivation and active efflux was excluded experimentally, target site modification appeared to be the most likely resistance mechanism.
Methods: Reduced drug binding to ribosomes and modification in 23S rRNA in the presence of Cfr was investigated by CMCT modification assays and primer extension analysis. The type of modification was identified by MALDI-TOF mass spectrometry. MIC testing of cfr-carrying strains followed the CLSI recommendations. Location of cfr on plasmids was shown by transfer experiments.
Results: In the presence of cfr, a reduced drug binding to ribosomes could be demonstrated for the phenicols chloramphenicol and florfenicol, the lincosamide clindamycin, the pleuromutilins tiamulin and valnemulin as well as for the streptogramin A antibiotic virginiamycin M1. MIC testing of cfr-carrying strains revealed in part dramatic increases in the MICs not only for the antimicrobial agents mentioned above, but also for the oxazolidinone linezolid. Analysis of the 23S rRNA revealed an additional methylation at position A2305 which is located in the overlapping binding area of phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A ($PhLOPS_A$) antibiotics. The gene cfr was mainly located on plasmids. Occasionally, it was integrated via insertion sequences IS21-558 into plasmidic copies of the fexA-carrying transposon Tn558.
Conclusions: 1) The gene cfr codes for a rRNA methyltransferase which targets the adenine residue at position 2305 in 23S rRNA. 2) Methylation at this position is believed to cause resistance by interfering with the correct positioning of the $PhLOPS_A$ antibiotics. 3) The gene cfr ist the first gene for transferable resistance to oxazolidinones and pleuromutilins. 4) Although this gene is currently detected very rarely among human and animal staphylococci, its location on plasmids points towards a potential for dissemination.

Determination of Modafinil (Provigil) in Plasma and Urine by High-Performance Liquid-Chromatography

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Modafinil (Provigil) is a new wake-promoting drug that is being used for the management of excessive sleepiness in patients with narcolepsy. It has pharmacological properties similar to that of amphetamine, but without some of the side effects associated with amphetamine-like stimulants. Since modafinil has the potential to be abused, accurate drug-screening methods are needed. Analytical methods are also needed for therapeutic drug monitoring and for pharmacokinetic studies. In this study, we developed a high-performance liquid-chromatographic procedure (HPLC) for the quantitative analysis of modafinil in plasma and urine. Provigil tablets (Cephalon, Inc.) containing modafinil were pulverized and extracted with methanol. After centrifugation, aliquots of the extract were used to prepare the urine and plasma calibrators. 3-Acetamidophenol and phenylthioacetic acid were used as internal standards for the analysis of plasma and urine, respectively. Modafinil was extracted from plasma and urine with ethyl acetate and analyzed on a C18 reverse phase column with methanol-water-acetic acid (500:500:1, v/v) as the mobile phase. Modafinil and the internal standards were analyzed at 220nm. The lower limit of sensitivity was 1.0 μg/mL, the within-day CV's were 4.8, 2.5, and 2.4-2.5 percent at 1.0, 5.0, and 10.0 μg/mL for plasma and urine, and recoveries from plasma and urine were 80.0 and 79.6%, respectively. Forty-nine two-hour post dose urine samples from sham controls or from individuals taking 200 or 400 mg of modafinil were analyzed without knowledge of drug administration. All 16 placebo urine samples were correctly classified as negative at a modafinil concentration <0.4 μg/mL. Except for one sample, all 32 two-hour post-dose urine samples tested positive. No interfering substances (> 0.4 μg/mL) were found in plasma samples from 28 randomly selected individuals or in urine samples from 16 individuals not taking modafinil. Likewise, aspirin and acetaminophen did not interfere with the HPLC method. Phenylthioacetic acid and modafinil acid, a major metabolite of modafinil, could not be extracted with ethyl acetate, but could be extracted from plasma and urine with ethyl acetate:acetic acid, 100:1, v/v.
Conclusions: Modafinil could be accurately and reproducibly analyzed in both urine and plasma. The HPLC method can be used for therapeutic drug monitoring, pharmacokinetic studies, and for drug abuse screening.

Bacterial Tetracycline Resistance: Prevalence, Evolution and Dissemination of Genes

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Background: Bacterial resistance to antibiotics is a global phenomenon. More than 40 distinct tetracycline resistance (Tc^R) genes confer resistance by four different mechanisms. Continually improving molecular and bacterial cultivation techniques have facilitated the identification of new genes in anaerobic bacteria. Recently discovered mosaic Tc^R genes appear to have arisen by homologous recombination between parental genes, simulataneously present in the same host bacterium. Mosaic genes are particularly prevalent in environments that have been exposed to tetracycline selective pressure. The first tet(W) gene was located on a large conjugative transposon, and intra-species gene transfer between bacteria is crucial in the spread of antibiotic resistance.
Methods: A macroarray screen containing 23 prevalent tetracycline resistance (Tc^R) genes was hybridized to DNA extracted from animal, human and soil samples. Sequences flanking specific Tc^R genes were compared. PCR amplification utilizing full-length and nested primer sets for different Tc^R genes assessed the prevalence and distribution of mosaic genes.
Results: The macroarray screen indicated that tet(W) is the most prevalent gene in gut ecosystems that are dominated by anaerobic bacteria. In contrast tet(W) was virtually undetectable in oral samples, which were instead dominated by tet(M). Analysis of sequences upstream and downstream of the tet(W) gene in diverse bacteria showed that short regions of 650bp and 100bp respectively are strongly conserved. This 2.7kb region may be a mobile mini-element, and may be, at least in part, responsible for the wide distribution of tet(W).
Mosaic genes (78%) outnumbered wild-type genes in samples obtained from commercial pigs, and constituted 50% of clones obtained from a human facal sample. Close analysis of mosaic genes combining tet(O) and tet(W) motifs illustrated that recombination hotspots exist. The most complex mosaic gene identified was tet(O/W/32/O/W/O).
Conclusions: 1) Different Tc^R genes dominate oral and faecal samples from the same individuals, reflecting the different microbial communities present 2) Identical mosaic genes have been identified by different research groups from different samples, illustrating their prevalence

Treatment of chronic myeloid leukaemia (CML) by imatinib mesilate in Togo

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Goal: Evaluate the efficacy of imatinib mesilate in chronic myeloïd leukemia (CML) patients followed in the Campus Teaching Hospital of Lomé.
Patients and Methods: Eighty patients followed since 55 months for CML in Lomé and using imatinib mesilate, supported by GIPAP Program, were investigated. BCR-ABL fusion was found in all them by RT-PCR technique in Créteil. Molecular follow up was based on BCR-ABL/GDR ratio.
Results: Eighty patients (6 females and 12 males) were included in the study. The mean age at diagnosis was 40.7 ±15.1 years old with limits between 18-70 years. All patients had splenomegaly at diagnosis. The average number of leucocytes/mm^3 was 187905 ± 75602.3 [55700-350000], the mean haemoglobin level was 9.4 g/dl ± 1.4 [5.7 and 11.5 g/dl]. Dose of imatinib administrated varied between 200 and 400 mg. On imatinib, the clinical remission was obtained on average 3 months [7 days-9 months], that of the haematological remission on average 3 months [20 days-14 months]. The long time obtaining haematological remission was due to the therapeutic breaks for major iatrogenic neutropenia. At the diagnosis, 33% of the patients were higher than 10^{-1} and 67% between 10^{-1} and 10^{-2}. To date, 5 patients (31.3%) have an undetectable transcrit (10^{-6}) since 11 months, 4 (25%) were between 10^{-3}-10^{-4}, 3 (18.7%) between 10^{-2} and 10^{-3} and 4 (25%) between 10^{-1} and 10^{-2}. Two deaths were noted after blastic transformation (due to very irregular administration of the treatment according to the induced cytopenia). In spite of a daily dose of 300 mg, the sensitivity to the treatment is higher than that described in the literature. Patients using imatinib in first intention was the best responders and presented less iatrogenic signs.
Conclusion: The imatinib mesilate in continuous treatment in the CML allows often early clinical, hematology and cytogenetic remission even in sub-Saharan Africa context.

Effect of Pectin-Papain Interactions on Thermo-Mechanical Properties of Pectin Films Applied for the Treatment of Skin Wounds

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Background: Pectin is a biodegradable and water-soluble polymer. In this work, pectin obtained from peels of the maracuya (Passion fruit) was applied. It was used as support for papain (EC 3.4.22.2) immobilization to make the films suitable for treatment of skin wounds with this enzymatic debridement agent. However, the mechanical properties of the films must be improved to obtain the material with higher resistance to break. In the present study the effects of pectin-papain interactions on enzyme stability and thermo-mechanical properties of films with and without glycerin (G) and polyvinyl alcohol (PVA) were evaluated.
Methods: Films were prepared using solution of 1 % pectin, 1 mg/ml of papain, G at 0.75% (v/v) or PVA at 0.25% (w/v). The films were studied by Differential Scanning Calorimetry (DSC) and Dynamic Mechanical Analysis (DMA), as well as in enzyme stability study performed spectrophotomerically using casein as substrate. Pectin-papain films with and without glycerin were tested on wounds of voluntary patients.
Results: Different glass transition temperatures (Tg) were detected: 18ºC for pectin system, 15.43º for pectin-papain, 18.48º for pectin-G-papain and 25ºC for pectin-PVA-papainsystem. The values of the tensile breaking for pectin and pectin-papain films were 9.16 and 8.88 MPa, respectively, with an elongation of 1.64% and 2.53%, while for pectin-G-papain films were 14.2 MPa and 9.99%, 11.2 MPa and 2.68% in the presence of PVA. The PVA (but not G) decreased papain activity and stability. Moreover, in assays performed on the voluntary patients treated with pectin-papain and pectin-G-papain films, healing of the wounds were accelerated without any negative secondary effects.
Conclusions: 1. Addition of plasticizers improves the mechanical properties of the pectin films. The comparison of thermo-mechanical parameters demonstrates the presence of interactions leading to a change of mobility of biopolymer chains. 2. Glycerin addition does not influence on papain-pectin film capacity to accelerate healing of the skin wound.

Variability of the Systemic Availability of Budesonide in Man when Administered Locally at Different Levels in the Gut with Different Doses of Ketoconazole

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Background: A number of studies have demonstrated that the gut mucosa contributes substantially to the overall first-pass metabolism of many drugs. The major active enzyme in these cases is cytochrome P450 3A4 (CYP3A4), which is expressed in the small intestine and the liver. The relative contribution of gut wall and hepatic metabolism is seldom known and there is also a lack of knowledge regarding to what extent different parts of the gastrointestinal tract differ with respect to metabolic activity. Aim: To investigate gut wall first-pass metabolism in various regions of the gut metabolism of budesonide, a CYP3A4 substrate.
Methods: Budesonide (3 mg) as a solution, was given locally in the gut on different occasions to eight healthy men, with and without pretreatment (5 min before) with a low dose of ketoconazole (10 mg) in solution to investigate the gut wall first-pass metabolism in jejunum, ileum, and colon. The solutions were infused through a thin, soft tube that was brought to the desired position by a peristaltically driven capsule. Simultaneously, deuterium-labelled budesonide, 0.2 mg, was given intravenously to make sure that body clearance of the drug was not affected by the low dose of ketoconazole (no hepatic inhibition). Budesonide in plasma was assayed up to 12 h by use of an LC-MS/MS method.
Results: Ketoconazole pretreatment increased systemic availability of budesonide 1.8 times after jejunal and 2.0 times after ileal infusion but it was virtually unaffected by ketoconazole after infusion into the colon. Terminal half-life (T½) and body clearance were similar after the treatments. The systemic availability of budesonide without ketoconazole pretreatment was 12, 16, and 13 % from jejunum, ileum, and colon, respectively.
Conclusion: Substantial CYP3A4 enzyme activity was indicated in jejunum and ileum while such activity was insignificant in the colon.

Neurotoxicity Related to Lithium Combination Treatment in a Patient with Schizoaffective Disorder

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Background: The therapeutic efficacy of lithium in the treatment of affective disorders is invaluable. Nevertheless neurotoxicity should be considered as a significant side effect. Neurotoxic encephalopathy has been described in the literature both in lithium mono-therapy with normal serum levels or with toxic levels and in combined treatment with other therapeutics, but particularly with neuroleptics. In the following the case of a patient with schizoaffective disorder who developed a neurotoxic encephalopathy related to lithium – risperidone combination treatment is explored.
Methods: A clinical case of encephalopathy is described. Neurological examination, psychopathological state and the results of EEG investigations are presented. The relevant theoretical considerations of the aetiopathogenetic mechanisms are discussed, differential diagnostic steps and therapeutic implications are described.
Results: A 60 year-old patient with schizoaffective disorder showed an acute neuropsychiatric state with severe cognitive deficits and an akinetic extrapyramidal syndrome under combined lithium risperidone treatment. An EEG investigation showed a marked change in basic activity. The clinical state slowly began to improve after withdrawl from the psychopharmaceutics, and the EEG also showed a clear improvement. The development of a neurotoxic encephalopathy under treatment with lithium and risperidone is the result of different aetiopathogenetic mechanisms. The most important hypothesis put forward is that lithium-neuroleptic treatment causes neurotoxicity by increasing dopamine receptor blockade which results in profound dopaminergic hypofunctionality reflected e.g. by extrapyramidal symptoms. A neuroleptic malignant syndrome can be said to be the most important differential diagnosis.
Conclusions: 1) Neurotoxic symptoms under lithium combination treatment may be interpreted as the interaction of different aetiopathogenetic mechanisms. Interactions in the dopaminergic system are very likely to play an important role. 2) The EEG is the most important diagnostic parameter for both the acute phase and for the follow up. 3) In patients developing signs of intoxication under lithium therapy, discontinuation of lithium medication should be considered.

Silkworm infection models to evaluate the therapeutic effects of antibiotics

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Most drug candidates obtained by in vitro screening are inappropriate as medicines due to their toxicity and their pharmacodynamics in the human body. Preclinical tests using animal models are essential, however, for evaluating the therapeutic effects of drug candidates for further development. Although mammalian models have been used to examine the pharmacodynamics of drug candidates, both the high cost and the ethical issues of sacrificing mammals for drug analysis can delay the development of potentially therapeutic drugs. The use of invertebrate animals for the evaluation of drug candidates can overcome these problems. We propose the use of silkworms, Bombyx mori, as model animals to evaluate the properties of drug candidates. The lower cost and smaller space required for the maintenance of silkworms compared to mice allows for a larger number of animals to be handled in limited facilities. Because of the long history of the silk industry, the methods for taking care of silkworms are well established. Silkworms are ideal for use in a large-scale drug screening system, as they are large enough to be used in injection experiments, for making hemolymph preparations, and for isolating organs such as the midgut, which are essential processes for studying the pharmacodynamics of drugs in individual animals. The silkworm fat body functions in drug metabolism, similar to the mammalian liver, and contains a number of cytochrome P450s and sulfur or glucose conjugation enzymes, which are involved in drug detoxification.
In this symposium, we report that pathogenic microorganisms, such as Staphylococcus aureus and Candida albicans, were lethal to silkworms, and clinically-used antibiotics had therapeutic effects in silkworms. Moreover, the effective doses of antibiotics in this silkworm infection model were similar to those in mammalian models. Further, the availability of antibiotics by oral administration, and the drug distribution and metabolism were similar between silkworms and mammals.

Inhibition of angiogenesis and melanoma metastasis by DisBa-01, an alphavbeta3-blocking RGD-disintegrin from Bothrops alternatus snake venom

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Background: The integrin □v□3 is involved in multiple aspects of tumor angiogenesis and metastasis, which makes this receptor a key target for the development of anti-cancer therapies. Snake venoms are natural sources of small integrin ligands named disintegrins, which act as selective integrin inhibitors. We have previously demonstrated that DisBa-01, a recombinant RGD-disintegrin from Bothrops alternatus venom glands, is a strong inhibitor of the □v□3 integrin, therefore inhibiting tumor cell adhesion to vitronectin (IC50 = 225nM for B16F10 melanoma cells).
Methods: Inhibition of angiogenesis: The ability of DisBa-01 to inhibit bFGF-induced angiogenesis was tested in a matrigel plug assay in athimic nude mice. Inhibition of lung metastasis: The anti-metastatic activity of DisBa-01 was evaluated by injecting the luciferase-expressing B16F10-2B8 cells mixed with DisBa-01 in the tail vein of C57BL6/j mice. The progression of pulmonary metastases was measured at day 1, 4, 7, 11 and 14 following cell inoculation by an imaging system. Flow assay: MDA-MB-231 cells labeled with cell tracker red were previously incubated with DisBa-01, mixed with whole blood labeled with calcein green and perfused at a shear rate of 1500 sec^{-1} in a flow chamber on a collagen type I-coated coverslip. Adhered platelets and cells were differentially counted using the software Image J.
Results: DisBa-01 dose-dependently decreased bFGF-induced angiogenesis in a matrigel plug assay (IC50= 83 nM). When injected intravenously to C57BL/6 mice together with B16F10 melanoma cells, DisBa-01 time- and dose-dependently inhibited lung metastasis. Under flow conditions, DisBa-01 (100nM) almost completely inhibited the adhesion of MDA-MB-231 cells to collagen I and to the extracellular matrix produced by endothelial cells as well. Deletion of the N-terminal up to 26 residues did not affect the inhibitory activity of DisBa-01 to the □v□3 integrin.
Conclusions: DisBa-01 is a potent new inhibitor of □v□3 integrin-dependent adhesion processes involved in tumor angiogenesis and metastasis.

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Correlation Between Clinicopathological Features and Allelic Loss at Tp53 In Metastatic Endometrial Cancer

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Adenocarcinoma of the endometrium (EC) is one of the most common malignancy of the female genital tract, with 4196 new cases diagnosed in 2005 in Poland. Although our knowledge of genetic features in primary endometrial carcinoma has been expanding, there is limited number of studies evaluated the role of molecular alterations during EC spread. Currently, we examined the allelic loss at the TP53 gene in 38 metastatic ECs, and investigated the relationship between LOH (Loss of Heterozygosity), p53 protein overexpression and clinicopathological features of cancer. Three intragenic TP53 polymorphic markers, located at intron 1, intron 4 and exon 4, were analyzed. Overexpression of p53 was evaluated immunohistochemically applying monoclonal mouse anti-human p53 antibody (clone DO-7, diluted 1:100) and the Vector Laboratories visualization systems. There was no significant association between LOH at intron 1 and clinical and pathological variables of cancer. A significant correlation existed between allelic loss at intron 4 and the presence of the neoplasm in the uterine cervix (R=0.319, p=0.049; Spearman rank correlation test). There was also a tendency for an inverse correlation between allelic loss at exon 4 and vascular space invasion, but this difference did not reach a significant value (R=-0.321, p=0.068; Spearman rank correlation test). p53 protein was overexpressed in 34% (13 out of 38) ECs, either in primary or in metastatic endometrial lesions, and was significantly related to patients' age (p=0.043) and to the presence of the neoplasm in the fallopian tube (p=0.046). Overexpression of the protein was significantly correlated with LOH at intron 1 of the gene (R=0,599, p=0.0001; Spearman rank correlation test), and a tendency existed for the correlation between p53 overexpression and allelic loss at intron 4/exon 4. Altogether, allelic loss at the TP53 is present in a subset of advanced-stage EC patients, and is correlated with p53 protein overexpression, particularly at intron 1of the gene.

In Vivo Molecular Imaging of Capromab Pendetide in Humans and Small Animals Using Combined Dual-Modality SPECT/CT and microSPECT/CT Systems

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Background: Capromab pendetide is a monoclonal antibody targeting an intracellular epitope of prostate specific membrane antigen (PSMA). This antibody radiolabeled with ^{111}In for radioimmunoscintigraphy is primarily used for detection of lymph node metastasis or recurrence of prostate cancer. The same antibody also has a potential as a radioimmunotherapeutic agent with a radiolabel that emits beta particles such as ^{177}Lu or ^{90}Y. Single photon emission computed tomography (SPECT) has been the choice of imaging radiolabeled capromab pendetide. In comparison to standalone SPECT, a combined dual-modality SPECT/CT technology can offer a) better image quality and quantitative accuracy of SPECT and b) means to localize uptake of radiolabeled capromab pendetide with anatomical details from computed tomography (CT). Over the past decade, we have improved methods to image both humans and animal models using the SPECT/CT technology and radiolabeled capromab pendetide.
Methods: Patient capromab pendetide imaging studies first started in 1999 using a prototypc SPECT/CT system at UCSF. Since 2003 the studies were performed using a commercial SPECT/CT system that has a lower-resolution CT capability. Most recently in 2008, we started patient imaging studies with a high-end SPECT/CT system with a diagnostic-quality 16-slice CT capability. We started imaging LNCaP xenograft mice using ^{111}In- and ^{177}Lu- labeled capromab pendetide using a dedicated small animal pinhole SPECT combined with CT system in 2005. We quantitatively analyzed interpreter's confidence level for patient data when SPECT/CT technology was used, and pharmacokinetics and biodistributions of ^{111}In- and ^{177}Lu- capromab pendetide in the xenograft models.
Results: We found an increased confidence level in interpretation of patient imaging data of ^{111}In-capromab pendetide using SPECT/CT over SPECT. ^{177}Lu-capromab pendetide showed similar or slightly better imaging characteristics over ^{111}In-capromab pendetide in the LNCaP models.
Conclusions: SPECT/CT imaging of capromab pendetide in humans improved the visual quality as well as our confidence in interpretation. The small animal imaging studies with both ^{111}In and ^{177}Lu as radiolabels showed a dual-role potential of ^{177}Lu-capromab pendetide as a radioimmunotherapy agent and as an imaging agent during radioimmunotherapy.

Illicit Drugs and Cardiac Arrhythmias in Athletes

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The current management of athletes with arrhythmias is complicated by the large use of "illicit drugs", taken, at any age, both by professional and non professional athletes.
The World Anti-Doping Agency (WADA) yearly updates a list of prohibited substances and methods banned by the International Olympic Committee. The list includes different classes of substances namely Anabolic Androgenic Steroids, Hormones and Related Substances (Erythropoietin; Growth Hormone, Insulin like Growth Factor, Mechano Growth Factors); Gonadotrophins; Insulin; Corticotrophins, Beta-2 agonists, Diuretics and other Masking Agents, Stimulants, Narcotics, Cannabinoids, Glucocorticosteroids Alcohol, Beta-Blockers and others.
The term "illicit drugs" comprises all categories of drugs banned by WADA, regardless of whether they are taken in order to improve physical performance (true "doping agents" or "performance enhancing drugs"), to mask the presence of specific doping agents during control tests ("masking agents"), or to counteract the hormonal side effects of doping agents.
Several illicit drugs may cause cardiac collateral effects, through a direct or indirect cardiac action, and may provoke especially arrhythmogenic effects, during short, medium or long term.
The cardiovascular effects comprehend a wide spectrum of diseases: hypertrophic, dilated, ischemic cardiomyopathies, myocarditis, thrombo-embolic diseases and also a wide range of supraventricular and/or ventricular cardiac arrhythmias, focal or reentry type, that are often symptomatic and potentially lethal even in healthy subjects.
The risk of lethal arrhythmias and sudden death (SD) is very high in subjects with preexisting cardiac diseases, particularly latent arrhythmogenic substrate or primary arrhythmic disorders including some inherited cardiomyopathies at risk of SD or with "ex novo" structural disease due to assumption of the illicit drugs. Together with a continuous effort in improving the analysis of prohibited drugs it is crucial that the doping control strategies include the investigation of mechanisms of cardiac action and toxic effects of every single drug in the current "2008" WADA list.
Particular attention has to pay to "recreational drugs" (or drugs of abuse) including ecstasy (MDMA) and other amphetamines and numerous very new synthetically derived formulations, several classified ad "designer drugs".

Detection Of Lamivudine And Adefovir Resistant Hepatitis B Virus Polymerase Gene Variants During Two Years Antiviral Theraphy Of Chronic Hepatitis B Patients In The South Of Turkey

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Background: Hepatitis B virus (HBV) belongs to the family of hepadnaviruses. The virus associated with acute hepatitis, chronic hepatitis, and development of hepatocellular carcinoma. Lamivudine (LAM) and Adefovir (ADV), are oral nucleoside/tide analogues, inhibit HBV replication and can markedly reduce serum HBV DNA levels and normalise alanine aminotransferase (ALT) levels associated with improvement in liver necroinflammatory activity, but the greatest drawback with LAM and ADV is the emergence of drug-resistant.
Methods: In this study, we have examined 80 sera samples obtained from patients with chronic hepatitis B ongoing LAM and/or ADV treatment in gastroenterology department for about 24 months. A 422bp part of HBV polymerase gene was amplified by a nested PCR protocol which including B, C and D domains of viral polymerase gene. This fragment was also sequenced by a DNA cycle sequencing protocol. The sequencing gel was visualized using silver-staining method.
Results: We have detected mutations related LAM resistance in HBV polymerase B and C domains in 15 sera samples (18.75%). Ten of 80 were M204I (12.5%), 3 of 80 M204V (3.75%) and 2 of 80 L179M (2.5%). We have also detected mutations related ADV resistance in B and D domains of HBV polymerase gene in 5 sera samples (6.25%). These mutations were 4 of 80 N236T (5%) and 1 of 80 P237H (1.25%).
Conclusions: The study reveals that antiviral therapy with viral polymerase inhibitors is still controversial and new antiviral strategies are necessary. This study is undergoing and planned to analyze all chronic hepatitis B patients ongoing lamivudine treatment in Mersin University research hospital.

The new biocidal agents with the high sporicidal efficiency

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Background: Permanent tendency of scientific workers in the area of development of the new disinfectants is to introduce the disinfection agents with the broad spectrum efficiency which are slightly or non toxic, have a good stability, they are biodegradable, easy applicable as the aerosols or as the foams. Using of foams as the carriers of chemical compounds with the disinfection efficiency has indisputable advantageous. The goal of this paper is to describe the results of the development of the two new biocidal agents with the high sporicidal efficiency.
Methods: At first the mixtures from different compounds were prepared. The first agents under the name HVĚZDA (STAR) contains as a efficacious component hydrogen peroxide and the other parts are alkalised mixture of tensides (quaternary ammonium salt and anionic tenside). The other of them whith working name PTSPCH is built-up from the hypochlorite sodium, other parts are partly derivate of pyrrolidone and partly organic phosphate. Both have alkaline character.Verified stability of HVĚZDA is one year and stability of the other agent was investigated for 200 days and continued till this time. Stability was verified partly using quantitative analysis concentration of biocids and partly microbiological inquire to sporicidal efficiency in agreement with European norm. It is assumed that both agents would be used in practice as bicomponente agent. Bactericidal, fungicidal and sporicidal efficiency were carried out in according to standard operational method. Bacterial strains from the Czech collection of microorganism (Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis) were used.
Results: The development of two new biocidal agents was successfully finished. Both have high disinfection effect particularly sporicidal efficiency.
Conclusions: We plan to verify these new agents in experiment with deactivation Bacillus anthracis spores in the special workplace in Czech republic.

Targeted Delivery of Cytotoxic Drugs by Means of Protein Vectors

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Background: Epidermal growth factor (EGF) and its receptor-binding fragment (EGFfr) were shown to be promising vectors for targeted delivery of cytotoxic agents to tumor cells. The use of short peptides as a vector components of the targeted preparations is more preferable owing to their high stability, easy availability and relatively low cost. The aim of the present study was design of vector peptides targeted to EGF receptor and investigating of cytotoxic activities of their conjugates with antitumor antibiotic doxorubicin (DOX).
Methods: Solid phase peptide synthesis (Fmoc strategy); study of growth-stimulating activities of peptides against fibroblast cell cultures; synthesis of DOX conjugates with peptides using crosslinking reagent; study of cytotoxic activity (CTA) of conjugates in vitro against human tumor cells.
Results: Two modified forms of EGFfr (EGFfr1 and EGFfr2) were synthesized. EGFfr1 differed from the original fragment by the presence of Ser instead of Lys in position 28. EGFfr2 contained Lys instead of Met in position 20 and Ser instead of Lys in position 28. Ser is one of the few amino acids forming the binding site in murine EGF. The undesirable conjugation at the ε-amino groups of Lys in the active center of the receptor-binding fragment can be avoided through substitution of Ser for Lys which, in its turn, prevents the inhibition of binding of the polypeptide to the EGF receptor. The substitution of Lys for Met_{20} in EGFfr2 was performed to improve the conjugation of the peptide to DOX. Both EGF fragments manifested biological activities in vitro which exceeded activity of native EGFfr. The conjugates manifested CTAs towards cultured human carcinoma cells HeLa which exceeded that of the free antibiotic 2-3-fold. The CTAs of the conjugates were close to that of free DOX against sensitive to this drug tumor cells $MCF\text{-}7^{Wt}$ and exceeded 1.5-2-fold the CTA of DOX against resistant cells $MCF\text{-}7^{AdrR}$ that characterized by hyperexpression of P170 protein.
Conclusions: 1) The amino acid substitutions in the EGFfr fragment are responsible for the increase of its receptor-binding ability. 2) Both peptides can be used for targeted delivery of DOX and, perhaps, some other antitumor drugs, to tumor cells.

Spectroscopic study on the interaction of the antitumoral drug emodin with bovine serum albumin: fluorescence, circular dichroism, SERS, SEF and stopped-flow techniques

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Background: Emodin shown an anti cancer effect in neuroectodermal tumor, breast cancer cells or prostate cancer. It binds Bovine Serum Albumin (BSA) forming a complex that is very important to ensure the drug delivery. Using Surface Enhanced Raman Spectroscopy (SERS), Surface Enhanced Fluorescence (SEF), Fluorescence, Circular Dichroism (CD) and Stopped-flow we have unequivocally characterized the binding sites of emodin/BSA complex.
Methods: SERS and SEF: U-1000 Jobin-Ybon Spectrophotometer provided by an argon ion laser (514.5 nm line). Fluorescence: Perkin Elmer 50B with an excitation wavelength of 295 nm. CD: JASCO-710 spectropolarimeter between 200 and 240 nm. Stopped-flow: Biological SFM-3 system coupled to a transmittance detector. The change of transmittance at 490nm (maximum absorption for emodin-BSA_f complexes)was monitored during the reaction time.
Results: From SERS and SEF we deduced that in complexes, the neutral and mono anionic drug species are predominant. From fluorescence and CD we calculate the binding constants. CD results indicate a change in the α-helical contents of the protein when binding occurs. Stopped-flow experiments indicate the presence of two different mechanism of reaction for the binding.
Conclusions: 1) The primary interaction site of emodin is Sudlow's site 2, where the bound drug presents a structure between neutral and mono anionic species due to the formation of some hydrogen bond. This interaction changes the α-helical contents of the protein. This process occurs for [emodin]/[BSA]≤2.0 ratios, and it implies a fast reaction with a complex mechanism of reaction, where the observed rate constant, k_{obs}, increases when [emodin]/[BSA] increases. After this interval, site 2 saturates.
2) The secondary interaction site of emodin to BSA occurs when [emodin]/[BSA]>2.0 and corresponds to Sudlow´s site 1 binding. Drug species binding to this site is not exactly the same than that binding to site 2, and it exhibits a form more displaced to the neutral one. This interaction does not change the α-helical contents of BSA. This process implies a slow reaction with a different mechanism, where the observed rate constant, k_{obs}, is invariable when [emodin]/[BSA] increases.

The impact of a mixture of doxycycline, an acid and a detergent on root canal débridement

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Background: Pulp and periapical pathoses are microbial in nature. Removal of this flora is essential for healing. While débridement of the root canal system is achieved by mechanical instrumentation, supplemented with chemical disinfection, removal of the smear layer (created by the mechanical instrumentation) and the adherent bacteria are not consistently achieved. Here we have combined doxycycline with citric acid and a detergent (MTAD) that reduces surface tension thereby allowing enhanced penetration of the solution.
Methods: Part I (smear layer removal): Forty-eight extracted human teeth were prepared. Sterile water or 5.25% sodium hypochlorite (NaOCl) was used as intracanal irrigant. Canals were treated with 5ml of one of the following final rinses: sterile water, 5.25% NaOCl, 17% ethylenediaminetetracetic acid disodium (EDTA), or MTAD. Presence or absence of smear layer and erosion of the root canal walls were examined under a scanning electron microscope. Part II (disinfection): Eighty-five extracted human teeth were contaminated with *Enterococcus faecalis*. After biomechanical instrumentation using 1.3% or 5.25% NaOCl, the teeth were exposed to a 5-min application of MTAD, 1.3% NaOCl, 5.25% NaOCl or a 1-min application of EDTA followed by 5ml of 1.3% or 5.25% NaOCl. Teeth or dentin shavings were cultured for presence or absence of test bacteria.
Results: Part I (smear layer removal): MTAD was more effective in removing the smear layer and did not significantly change the structure of the dentinal tubules. Part II (disinfection): Fisher's exact test showed that combination of 1.3% NaOCl and MTAD was significantly more effective in disinfecting the root canal system than the other regimens. Chi2 test showed no difference among the other regimens.
Conclusions: Modification of doxycycline with citric acid and a detergent allows effective disinfection of the root canal system after short exposure. This contact disinfection might be partly due to acidity of the solution. Furthermore, the smear layer is removed without significant alteration to the dentin structure thereby enhancing the débridement process.
Authors' disclosure statement: The authors acknowledge that, as an institution, Loma Linda University retains a financial interest in this product.

Drug Cell Interaction at Molecular Level

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Background: We are using Patch clamp technique to measure the ionic currents involved in neuromuscular disorders and are looking at the drug cell interaction at the molecular level taking muscular dystrophy as an example. Many forms of muscular dystrophy are associated with a structural fragility of the muscle membrane, whereby membrane damage exceeds the ability of muscle to repair itself, resulting in the progressive degeneration of muscle fibers. Clinically and genetically, they are a heterogeneous group of inherited diseases. Mutations in several individual genes are now known to underlie the pathogenesis of different types of muscular dystrophy. Some of these gene mutations involve proteins expressed throughout the body, yet appear to preferentially affect skeletal muscle.
We are interested to detect association of acetylcholine receptor protein with voltage gated sodium currents in biopsied cultured cells.
Methods: Whole cell and single channel configurations of patch clamp technique were used to measure the ionic currents gated by nicotinic acetylcholine receptors using acetylcholine and non depolarizing neuromuscular agent tubocurarine along with sodium channel agonist veratridine.
Results: Patient 1, a 6-year-old boy, had severe myasthenic symptoms since infancy. Patient 2 was a 44-year-old man. Both used wheelchairs and had a 30-50% EMG decrement on 2-Hz stimulation. Evoked quantal release was reduced to approximately 25% of normal in both. In Patient 2, the synaptic response to acetylcholine was further compromised by degeneration of the junctional folds with concomitant loss of the acetylcholine receptor (AChR). Patch clamp study of the muscle biopsy cultured cells confirmed the depletion of nicotinic receptor channel activities and sodium channel agonist veratridine increases the acetylcholine gated currents.
Conclusions: Combined clinical and in vitro electrophysiological findings define two types' congenital myasthenic syndromes gated by acetylcholine that can be regulated by sodium channel agonist veratridine.

Bacterial Drug Efflux Pumps: Significance for Antibiotic Resistance and Pathogenicity

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Background: The development and use of chemicals with antibacterial properties revolutionized the treatment of infectious diseases, but mechanisms of bacterial resistance to many antibacterials have evolved and threaten public health. Interestingly, the acquisition of resistance genes often decreases bacterial fitness in vitro, but little definitive information is available regarding their impact on bacterial fitness during infection. We examined this problem with the human pathogen Neisseria gonorrhoeae and an experimental murine infection model and tested if regulation of a multi-drug efflux pump can impact bacterial fitness.
Methods: This study employed a murine vaginal infection and strains of N. gonorrhoeae bearing defined mutations in the mtrR or mtrA genes; mtrR encodes the MtrR repressor and mtrA encodes the transcriptional activator of the mtrCDE operon. Fitness differences were determined by co-infection of mice with wild type and mutant strains. Promoter mapping was performed by primer extension analysis and mutations were identified by DNA sequencing.
Results: We determined that transcriptional regulatory mutations that de-repressed efflux pump gene expression increased levels of gonococcal resistance to antimicrobials and fitness in vivo but not in vitro. These mutations mapped to the transcriptional repressor gene mtrR, a 13 bp inverted repeat sequence within the mtrR promoter and at position -120 upstream of the mtrCDE operon; the latter gives evidence of generating a new promoter element. In contrast to mutations that de-repressed mtrCDE gene transcription, a null mutation in the mtrA gene decreased gonococcal fitness in vivo, but this could be counteracted by second site mutations that de-repressed mtrCDE expression.
Conclusions: The MtrC-MtrD-MtrE efflux pump produced by gonococci is similar to other efflux pumps possessed by Gram-negative bacteria. We propose that this pump is required for gonococcal proliferation at mucosal sites and levels of its expression can significantly impact both antibiotic resistance and in vivo fitness.

Cytogenetic Response of Imatinib Mesylate in Chronic Phase Chronic Myeloid Leukemia

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Background: The chronic myeloid leukemia (CML) is characterized by presence of Philadelphia Chromosome (Ph) in more than 90 percent of cases. The reciprocal translocation between chromosomes 9 & 22 results in the formation of Ph chromosome and a chimeric BCR-ABL fusion gene that causes CML.The disease follows a triphasic course – initial chronic phase (CP), accelerated phase (AP) and terminal blast crisis phase (BP). Imatinib mesylate (Glivec®, Gleevec™, formerly STI571), a selective tyrosine kinase inhibitor, decreases the number of BCR-ABL positive colony formation in CML patients. Aims: 1) To perform chromosomal analysis in CML patients. 2) To determine the cytogenetic response of Imatinib in Ph positive CML patients in chronic phase (CP).
Materials and Methods: This study was carried out in the Cytogenetic Laboratory at BP Koirala Institute of Health Sciences, Dharan, Nepal. Fifty clinically diagnosed and hematologically proven CML patients in chronic phase were selected for bone marrow aspiration at the time of diagnosis for chromosomal analysis. Those who were Ph positive received Imatinib mesylate 400mg orally daily. The follow up chromosomal analysis was done after six to twelve months of Imatinib therapy to monitor the size and progression of the Ph positive clone. Cytogenetic studies were performed by GTG banding techniques. Karyotypes were interpreted according to the 1995 International System of Human Cytogenetic Nomenclature. Cytogenetic responses (CRs) were determined by the percentage of metaphase cells that were negative for the Ph chromosome.
Results: Out of 50 CML-CP patients 48 (96%) were Ph positive and 2 (4%) were Ph negative at the time of diagnosis. Out of 48 Ph positive patients who were under Imatinib treatment, 25 patients were available for follow up. After a median follow up of 12 months, 16 (64%) patients had major cytogenetic response (Ph+ve cells less than 35%)[partial CR (Ph+ve cells more than 0% to 35%) in 15 (60%) and complete CR (Ph+ve cells 0%) in 1(4%)], 7 (28%) patients had minor cytogenetic response (Ph+ve cells 35% to 65%) and 2 (8%) patients showed Imatinib resistance.
Conclusion: Imatinib reduced Ph chromosome in 23 (92%) out of 25 newly diagnosed Ph positive CML patients in chronic phase.

Intragraft Administration Of Abciximab And Verapamil Combined With Direct Stenting Prevents Slow-Flow And No-Reflow Phenomenon In Saphenous Vein Graft Percutaneous Coronary Intervention

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Background. Slow flow/ no-reflow phenomenon (SF-NR) complicates up to 15% cases of percutaneous coronary intervention (PCI) in saphenous vein grafts (SVG). We hypothesized that a strategy of prophylactic intragraft administration of abciximab and verapamil into the SVG, combined with immediate direct stenting of the graft lesion without pre-dilatation, would reduce the risk of platelet activation, microvascular vasospasm and distal plaque embolization respectively and cause a reduction in the incidence of SF-NR.
Methods. Data from 130 consecutive patients who underwent PCI of SVG lesions in a single center over a 7-year period were reviewed. Patients who underwent conventional PCI technique (balloon pre-dilatation of the target lesion prior to stent deployment; optional use of intragraft verapamil or intravenous abciximab) were assigned to the control group (n=72). The patients who received prophylactic intragraft administration of abciximab (0.25 mg/kg) and verapamil (100-300 mcg, depending upon blood pressure and heart rate) through the guiding catheter followed by direct stenting were assigned to the novel strategy group (n=58). The primary outcome was the occurrence of SF-NR Clinical endpoints included death, MI, target vessel revascularization (TVR), and MACE during the hospitalization period, 30 days and at 1 year.
Results: SF-NR occurred more frequently in the control group compared to the novel strategy group (11% vs. 2%, P=0.04). One patient in the control group died after developing persistent SF-NR and acute MI post-PCI. No death was reported in the novel strategy group. Three patients, all in the control group, developed post-PCI MI during the index hospitalization. The difference in 30-day MI and TVR rates did not reach statistical significance. There was a non-statistically significant trend towards higher 1-year MI rate in the control group (8% vs. 2%; P=0.13). The control had significantly higher rates of MACE (25% vs. 7%, P=0.01) and TVR (22% vs. 7%, P=0.03) at 1 year as compared to the novel strategy group.

Substance Use and Its Functions, Dysfunctions, and Alternative Functions in Contemporary Society

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Background: People use and produce substances with some purposes; substance use exists and persists for some reasons; and substance users fit into society and fulfill some functions for it. Aims: 1) To identify various functions substance use fulfills in contemporary society. 2) To identify various dysfunctions substance use has in contemporary society. 3) To identify various alternative functions substance use serves in contemporary society.
Methods: This study is based on general literatures in the humanities and social sciences. Major research methods used range from classification, categorization, correlation, comparison, and logical reasoning to theoretical argumentation. For example, contrasting categorization distinguishes manifest, material, short-term, and peripheral from latent, moral, long-term, and core functions or dysfunctions.
Results: Substance use has both functions and dysfunctions. Specifically, functions or positive effects include pain alleviation, symptom management, stress control, socializing, exchange, trade activities, service provision, and job creation. Dysfunctions or negative consequences involve dependency, withdrawal syndrome, social vice, crime, black market, waste of social resources, and drain on the taxpayers' money.
Conclusions: 1) Substance improves human adaptation to nature. 2) Substance use and abuse act as a substitute for more serious deviance and even crime in a society. 3) Substance users serve their group, culture, and historical era as messengers of critical issues or innovators of alternative lifstyles.

POSTIRRADIATION activation of LINE DNA Retroposition in realization of the adaptive response

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mobile LINE, resistance, retroposition

There is a set of concepts of an important role of the mobile genetic elements in particular LINE (long interspersed) DNA in adaptation to the variable environment. However, small molecular mechanisms of resistance are not clear. So an issue of biological significance and origin of nucleic acids of blood plasma as an indicator of extreme states arises.
To study the role of LINE retroposition, a rat model of the radiation stress was used. The dependence of dynamics of the contents of 5'- and 3'-end fragments of LINE and high-molecular (hm) and low-molecular (lm) DNA on the irradiation dose in blood plasma and application of radio protector (WR-2721) was studied. Dynamics of the contents of LINE 5'-end fragments and [3H]-thymidine, included in DNA of the liver, spleen and thymus cells of irradiated animals was also examined. The methods of PCR and PAAG electrophoresis were used.
Unequal reactions of 5'- and 3'-end fragments of LINE to the radiation dose were revealed, pointing to the postirradiation activation of retroposition process. The degree of this activation nonlinearly depends on the dose. At the sublethal doses, LINE retroposition was growing with increasing in irradiation dose, whereas at minimum absolute lethal dose it was partially inhibiting. Dynamics of the separate fractions of extra cellular DNA 2 - 5 h after irradiation was defined by decreasing amount of hmDNA and increasing amount of lmDNA.
Influence of radio protector on the irradiated animals was expressed by increasing in the speed of clearance of hmDNA, not accompanied by accelerating the accumulation of lm DNA of blood plasma. This result shows the role of hmDNA capture by the cells. The changes of LINE retroposition parameters were similar to that of hmDNA in these conditions. It indicates the involvement of the horizontal transfer of genes in mechanisms of radio protective effect.
The revealed drift of 5'-end fragments of LINE and [3H]-thymidine included in DNA of the liver, spleen and thymus cells suggests the involvement of the mechanism of LINE retroposition in the coupling of functions of immunocompetent tissues, important for the adaptation.
In conclusion, the dynamics of LINE retroposition parameters in the blood plasma (5'- / 3'-end fragments ratio) reflects the efficiency of adaptive systems and injury severity of organism in extreme states.

Screening Phage Genomes to Identify Novel Antimicrobial Targets in Mycobacteria and Related Organisms

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Background: Abundance of phages in nature and enormous diversity in their genetic makeup suggest the presence of a huge number of unknown bactericidal mechanisms and can be exploited to identify potential susceptibility targets in drug-resistant bacteria. Aim: 1) To discover genes from novel phages whose products inhibit Mycobacteria and related organisms. 2) To elucidate their mechanisms of action.
Methods: Phages plaquing on Rhodococcus were isolated from soil and purified by CsCl gradient centrifugation. Their morphologies were determined by transmission electron microscopy and genome sizes by pulsed-field electrophoresis. Genomic libraries were constructed using an E. coli – Rhodococcus shuttle vector pDA71. Library clones were individually screened by transformation into the host and Mycobacterium. Inhibitory DNA fragments were subcloned to determine minimum necessary DNA which were then sequenced.
Results: Four phages plaquing on Rhodococcus were isolated. All belonged to the Siphoviridae family and possessed genomes of 39kb to 140kb. Out of 80 randomly selected clones from their genomic libraries, 21 were inhibitory to R. erythropolis, as they had transformation efficiencies of $< 10^2$/µg DNA compared to 2×10^4/µg DNA for the vector-only control. Of 9 inserts selected for subcloning, 5 also inhibited M. smegmatis. Inhibitory DNA from the subclones revealed 18 open reading frames, 13 of which had no sequence similarity in databases. ORFs with sequence similarities were to thymidilate synthase complementing protein, HNH endonuclease, capsid protein, tail-related protein, and α subunit of DNA polymerase III.
Conclusion: Isolated phages were shown to provide a large pool of novel antibacterial genes. ORFs with sequence similarities have shown the promise of this approach, as they suggested potential interference with the biosynthesis of dTMP, DNA and protein, and the integrity of DNA and plasma membrane.

Treatment strategies for multiple liver metastases from colorectal cancer

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Background: As extended hepatectomy (Hx) and chemotherapy including targeted agent has developed during the last several years, the treatment outcome for multiple liver metastases from colorectal cancer has implored.
Aim: To clarify the current status of Hx with chemotherapy.
Method&Results: Extended Hx 87 patients who underwent Hx for multiple (>4) and bilobar liver metastases from colorectal cancer from 1992 to 2006 were enrolled in this study.
Results: The mean resected volume of straightforward Hx in 51 patients, Hx after portal embolization (PE) in 13 and two-stage Hx with or without PE in 23 was 401.9g, 654.5 and 879.9 individually. There was no mortality nor significant difference of morbidity among them. The hypertrophy ratio of the future remaining liver volume (before/ after the first procedure) in Hx after PE, two-stage Hx and two-stage Hx with PE was 1.25, 1.25 and 1.6 individually. The 5-year overall survival rate and 5-year disease-free survival rate after Hx were 38.0% and 11.7% individually. From these results, when the remaining cancer-free liver volume is only 200gm, two-stage Hx with PE should be employed. Perioperative chemotherapy
Methods: The treatment consists of systemic chemotherapy with 5FU+FA+CDDP or currently FOLFOX4 and hepatic artery infusion (HAI) with 5FU+FA+CDDP.
Results: 5-year survival rate of adjuvant systemic chemotherapy (49.9%) or adjuvant HAI (54.8%) was significantly higher than that of the absence or less than 5g of total injected 5FU through the HAI. There was a significant difference in survival of patients with 6 or more tumors and CEA > 11ng/ml between patients with and without neoadjuvant chemotherapy. In the 5-year overall survival rate of the responders consisting CR, PR and ST to the chemotherapy (48.8%) was significantly higher than that of the non responders (22.6%). Downstaged chemotherapy provided 5-year survival rate of 34.7% after Hx in 19 out of 138 patients (13.8%) with initially irresectable liver metastases.
Conclusion: Alternation therapy of potent chemotherapy and extended Hx enabled patients with multiple bilobar liver metastatis to survive a long period, specifically for patients of which tumor showed responsiveness to chemotherapy.

Serum Alcohol Dehydrogenase: A Sensitive Biomarker of Ongoing Graft Function After Liver Transplantation

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Background: In the postoperative management of liver transplantation, it is essential to monitor the graft status on a real-time basis to ensure the early diagnosis of postoperative complications. Currently, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, lactate, and other markers are used for that purpose. Alcohol dehydrogenase (ADH) is an enzyme specifically located in the cytoplasm of hepatocytes. The purpose of this study was to assess the potential usefulness of serum ADH activity as a biomarker of graft function following liver transplantation.
Methods: Blood samples were obtained from 26 patients who underwent living-donor liver transplantation. Serum ADH activity, ALT, AST, total bilirubin, and lactate were evaluated for 3 weeks after transplantation in each case. In our hospital, the reference range of serum ADH activity is $\leq$ 4 IU/l (37°C).
Results: In patients without any postoperative complication, serum ADH activity normalized at 2.9±1.2 days. Values of serum ADH activity were remarkably elevated in patients with vascular complications, whereas they were only slightly elevated or remained within the reference range in patients with acute cellular rejections. In vascular complications, serum ADH activity peaked prior to elevation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and once the cause of damage was resolved, the values returned to reference range more quickly than did ALT and AST.
Conclusions: ADH has a lower molecular weight and a shorter blood half-life than ALT and AST. This can explain how ADH is released into the blood earlier than ALT and AST and normalizes more rapidly after the cause of liver damage is removed. ADH is abundantly distributed in the centrilobular area in the liver. Therefore, ADH shows extremely high values in those conditions that cause severe damage to the centrilobular area, such as blood flow decrease.
In conclusion, monitoring serum ADH activity in addition to ALT and AST may provide more sensitive ongoing graft status and valuable information for the differential diagnosis of vascular complications and acute cellular rejection.
Authors' disclosure statement (not counting towards the character count)**:**
This section is NOT required. If authors wish to disclose any relevant information, please include this information in brief here.

A Bevacizumab, An Anti-VEGF Antibody, As A MAGIC BULLET for Retinal Disease?

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Background: Recently, many ophthalmologists who specialize in retinal diseases have interests in macular edema (ME) which directly leads visual disturbance. Macular edema was often seen in vasoproliferative and ischemic retinal diseases as diabetic retinopathy (DR) and retinal vein occlusion (RVO), and which pathogenesis are related with VEGF expression, therefore anti-VEGF therapy is expected to reduce ME. In this presentation, the effectiveness of intravitreous injection of bevacizumab, an anti-VEGF antibody against DR- and RVO-associated macular edema was evaluated.
Methods: 86 eyes of DR-associated ME and 75 eyes of RVO-associated ME participated in this study. After the initial ocular examination, 1.25 mg of 0.5 ml bevacizumab was injected intravitreously in each eye. ME was evaluated by measuring macular thickness with optical coherence tomography (OCT) for up to 24 weeks. Clinical course of best corrected visual acuity (VA) in each eye was monitored.
Results: Before starting this study, averaged macular thickness was 634.6 ± 96.5 □m in DR-ME, and 662.1 ± 104.8 □m in RVO-ME, which is no statistical difference between two groups. After the injection, averaged macular thickness in both diseases showed prominent decrease to 63.5 ± 14.2 % in DR-ME and 40.7 ± 11.6 % in RVO-ME within 1 month. However 70 eyes of 86 (81.3%) DR-ME and 49 of 75 (65.3%) eyes of RVO-ME showed recurrence of ME by 24 weeks. There is no significant difference of initial VA between both groups. After the injection, VA in both groups showed rapid and temporary improvement and after that it gradually decreased but kept significant improvement at 24 weeks. Temporal regression of VA in RVO-ME eyes was significantly better than that of DR-ME eyes.
Conclusions: Intravitreous injection of bevacizumab is possible to reduce macular edema, however, recurrence of the edema was observed in most cases. Although the other therapies including retinal photocoagulation or vitreous surgery must be added, the rapid and prominent reducing effects by intravitreous bevacizumab prevent visual dysfunction which is reflected by continuous macular edema. The effect of bevacizumab against macular edema was more expected in RVO than in DR.

Prospective Magic Bullet Cobalt: Its Effect, Mode of Action and Utility for Hypoxic Study

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Background: Ascent to high altitude results in the risk of altitude sickness including high-altitude cerebral edema (HACE). HACE is the most severe form and if no intervention is implemented, death may result. Aims: To evaluate effect of cobalt chloride ($CoCl_2$) supplementation on 1) Hypoxic tolerance and facilitation of acclimatization to hypobaric hypoxia (HH), 2) HH induced oxidative stress, 3) HH induced vascular leakage, 4) global gene expression with and without HH in rat brain
Methods: Male Sprague Dawley rats supplemented with $CoCl_2$ (12.5mg Co/kg b.w., oral) for 7days were subjected to 32,000 (till gasping) and 25,000 ft (48hrs) for tolerance and acclimatization/oxidative stress studies respectively by monitoring gene expression and various biochemical parameters. Cerebral vascular leakage and edema were monitored by radiometric and histological analysis after subjecting the rats to an altitude of 30,000ft (5 hrs). Global gene expression was studied by microarray. P<0.05 was taken to be significant.
Results: $CoCl_2$ improved hypoxic tolerance via increased expression of hypoxia inducible transcription factor (HIF-1) and its regulated genes. Administration of $CoCl_2$ appreciably attenuated the free radical (ROS) generation, oxidation of lipids and proteins and maintained GSH/GSSG ratio after HH similar to that of control rats by induction of HO-1 and MT levels via HIF-1 signaling mechanisms. $CoCl_2$ preconditioning inhibited HH induced vascular leakage by lowering NFκB activity and its regulated pro-inflammatory mediators. This is shown to be mediated by cobalt-induced reduction in ROS/NO and increase in HO-1 and MT. A battery of core genes was identified by microarray; a few genes were confirmed by RT-PCR and immunoblotting. Pathway predictions were also performed using KEGG software highlighting certain pathways affected by these treatments.
Conclusion: 1) $CoCl_2$ improves hypoxic tolerance and facilitates acclimatization to HH, 2) It attenuates HH induced oxidative stress, 3) It attenuates vascular leakage induced by HH, 4) A number of endogenous molecular mechanisms studied may explain how preconditioning protects against deleterious effects of hypoxia and may provide novel therapeutic targets for treatment of cerebral edema.

One hundred years of Paul Ehrlich's Chemotherapia specifica: How to get it for effective and safe cure of cancer patients

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Background: Both neoadjuvant and adjuvant chemotherapy, being carried out so far with cytotoxic agents, single or combined, are highly toxic to the patient because they distribute in the whole body. The lifelong experience of Paul Ehrlich for finding chemotherapia specifica agents had been based mainly on: i) healthy nutrition; ii) avoiding disease risk factors in life-style; iii) study of the cause of cancer; iv) finding a suitable non toxic pharmaceutical or radiopharmaceutical which can eliminate the cause of cancer. Epidemiological and etiological results for the incidence of different cancers are of great help in finding the cause of cancer.
Methods: We determine the total-body distribution of radiopharmaceuticals of known chemical composition, and study trace elements in the disease and also in the neighbouring healthy tissue with sector-field inductively coupled plasma mass spectrometry (SF-ICP-MS).
Results: Our study have shown that radiopharmaceuticals for cancer diagnosis and therapy are continuously changing their chemical composition after synthesis. They are therefore of little use for diagnosis and therapy. We are, at present, trying to develop chemotherapia specifica for safe and effective cure of bladder cancer patients. The risk factor for bladder cancer in the order of their decreasing harm are: i) cigarette smoking, ii) red meat consumption, iii) alcohol drinking, iv) coffee drinking.
Conclusions: The bladder cancer patients have to avoid these injurious substances as soon as possible when early signs of bladder cancer are observed. We have found how these risk factors work together to cause bladder cancer

Vaccine-derived polioviruses (VDPVs) - a grey cloud around a magic bullet

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Background Poliomyelitis has nearly been eradicated. The number of endemic countries has been reduced to 4 world-wide since the 1950's by use of inactivated and oral polio vaccines (IPV and OPV, respectively). Surveillance programs are an essential component of eradication programs that insures that all chains of transmission have in fact ceased. Israel has been poliomyelitis free since 1988.
Methods Since 1989, Israel has employed routine monthly sewage surveillance at >15 sites to monitor for presence of non-vaccine poliovirus. Sampling sites cover at least a third of the Israeli population.
Results 34 highly diverged [8 to >15%], neurovirulent in a transgenic mouse model, type 2 vaccine derived polioviruses have been periodically isolated from sewage in the greater Tel Aviv area [pop. 1800000] over the past 10 years. Molecular analyses strongly suggest that the isolates represent two major lineages originating from two persistently infected and continuously excreting individuals and or a small group of their contacts. By moving sampling sites up the sewage system, the population that contain each of the excretors has been reduced to 50,000 and 350,000.
Conclusions 1. To meet the challenge of eradication, the number of persistently infected individuals globally should be estimated. 2. High vaccination coverage must be maintained as long as persistently infected individuals continue to excrete. 3. OPV should be replaced by a non-live vaccine as soon as feasible to prevent new persistent infections.

When "Magic Bullets" Cause Collateral Damage (SJS/TEN and AGEP)

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Background: A "magic bullet" drug is supposed to have a desired therapeutic effect ideally without side effects. Yet unwanted reactions to drugs are very common in clinical routine. Licensing procedures – from preclinical data to pase III clinical trials - try to detect the risk of drugs for unwanted effects before they get on the market. Yet some reactions are too rare to be noticed within such trials. Typical examples for this are severe cutaneous adverse reactions like the Stevens-Johnson syndrome (SJS) / toxic epidermal necrolysis (TEN) spectrum (incidence 2-3/Mio inhabitants/year) and acute generalized exanthematous pustulosis (AGEP), Due to their severity (mortlity rate for TEN is still more than 30%) and sequelae they cause a severe burden to patients and the healthcare system.
Methods: For more than a decade an international study group collected and investigated a large number of patients with severe cuatneous adverse drug reactions in a series of pharmacoepidemiological studies. In the EuroSCAR study relative risks for different drugs to cause SJS/TEN and AGEP could be calculated in a case control setting.
Results: Strong associations with the occurrence of SJS/TEN could be confirmed for anti-infective sulfonamides, allopurinol, carbamazapine, phenobarbital, phenytoin, and oxicam-NSAIDs (allopurinol being the most common cause of these reactions within our network). Among drugs recently introduced into the market, associations were significant for nevirapine and lamotrigine, and weaker associations for sertraline, pantoprazole, and tramadol.
For AGEP strongly associated drugs, i.e. drugs with a lower bound of the 95% confidence interval (CI) of the odds ratio (OR) > 5 were pristinamycin, ampicillin /amoxicillin, quinolones, (hydroxy)chloroquine, anti-infective sulphonamides, terbinafine, and diltiazem.
Conclusions: Different drugs have a different risk of causing SCARs. The spectrum of drugs is also different for diferent type of unwanted reactions. Due to the rarity of such events their risk i soften not detected in the premarketing phase which emphasizes the importance of pharmacovigilance activities. Apart from surveliillance, research has o be focussed on pharmacogenetics to reduce the risk of these life threatening unwanted drug effects in the future.

A New Strategy to Inhibit Foam Cell Formation in Atherosclerosis Using the Soluble Immunoadhesion CD68-Fc

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Background: Despite their potent lipid-lowering effects, the systemic use of statins is limited by side effects. To establish a new strategy for the inhibition of foam cell formation in a highly specific manner at the site of plaque formation, we cloned a fusion protein consisting of the extracellular domain of the scavenger receptor CD68 and a human Fc domain (CD68-Fc). The aim of this study was to evaluate the binding properties and the antiatherosclerotic potential of CD68-Fc.
Methods: For binding studies, an ELISA assay against the human Fc-fragment was used on human carotid plaque pieces sticking to culture plates. In vivo the binding of radioactively labeled CD68-Fc (^{124}JCD68-Fc) was studied using ApoE$^{-/-}$-mice which develop lipid rich-atherosclerotic lesions within 20 weeks. In vitro, effects of CD68-Fc were studied in our atheroscreen model in which cocultivation of human platelets with CD34^{+}-progenitor cells for 5-10 days induces foam cell formation. Finally, CD68-Fc uptake kinetics of Dil-Ox-LDL was performed at monocyte/macrophages.
Results: Stainings and ELISA with Fc-antibodies showed that binding of CD68-Fc at lipid-rich human plaques was significantly higher compared to control-Fc. After intravenous injection of ^{124}J-CD68-Fc, specific binding could also be detected by autoradiography in contrast to wildtype-mice without atherosclerotic lesions. In vitro, laser scan microscopy showed that the foam cells express the scavenger receptor CD68 and that they take up labelled OxLDL. Cell counting and confocal microscopy demonstrated that foam cell formation could be prevented efficiently by CD68-Fc. Furthermore, CD68-Fc inhibited matrix metalloproteinase-9 activity, an important function of foam cells. Finally, FACS-analysis provided proof that the underlying mechanism of CD68-Fc is a reduction in cellular uptake of modified lipoproteins.
Conclusions: This new fusion protein, a soluble form of the human scavenger receptor CD68, specifically bound to foam cells in vitro and to lipid-rich plaques in vivo with high affinity and attenuated foam cell formation. Thus, CD68-Fc seems to be a promising new tool to prevent foam cell formation or to transform vulnerable lipid-rich plaques into stable fibrous plaques with reduced lipid content in patients with advanced atherosclerosis.

All abstracts are listed in alphabetical order of the presenting author.

Application of the Fluorescence Quenching Method to the Study of the Interaction of the Anti-psychotic Drug Chlorpromazine with Serum Albumin

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Background: The proper understanding of drugs binding to plasmatic proteins is an important pharmacological parameter determining their distribution, absorption and elimination, with possible undesirable clinical effects The aim of this work is to study the binding of chlorpromazine (CPZ) with human and bovine serum albumin (HSA and BSA). CPZ is a neuroleptic still applied in psychosis treatments and its chronic use may cause severe side effects, such as parkinsonism and tardive diskinesy. Here we present the Stern-Volmer constants, besides discussing the binding process.
Methods: Intrinsic fluorescence of BSA and HSA were measured by selectively exciting their tryptophan residues at 290nm. The gradual quenching was observed by the titration of both proteins with CPZ, and the Stern-Volmer graphs were plotted to evaluate the quenching constants.
Results: HSA titrated by CPZ yielded a linear Stern Volmer plot, whereas BSA yielded a slightly concave curve upward when CPZ concentration was higher than 10 □M. Comparison of experiments with HSA carried out at 35º C and 25º C revealed an important deviation in the slope of the Stern-Volmer plot. For HSA, the Stern-Volmer constants were $4.1x10^4$ ($\pm1.0x10^2$) M^{-1} at 25ºC, and $6.7x10^4$ ($\pm2.0x10^2$) M^{-1} at 35°C. The increasing of the angular coefficient caused by the higher temperature suggests the occurrence of dynamic quenching for HSA. The estimated constant for BSA was $3.3x10^4$ ($\pm9.0x10^2$) M^{-1} at 25ºC.
Conclusions: (1) CPZ quenches the fluorescence of HSA by collisional quenching, interacting with this protein without forming complex. (2) The primary binding site of CPZ is close to the single tryptophan residue of HSA, at the position 214, in the hydrophobic region of the chain. (3) CPZ binds to BSA near to the tryptophan residue located at the position 212.

The Proarrhythmic Potential of Macrolides: The Role of Interactions. A Review

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Background: Several antiarrhythmic and non-cardiovascular drug therapy, including antimicrobial agents have been implicated as a cause for QT interval prolongation, torsades de pointes (TdP) ventricular tachycardia and sudden cardiac death. Most of the drugs that have been associated with lenghtening of the QT interval or development of TdP can also block the rapidly activating component of the delayed rectifier potassium current (IKr) in the ventricular cardiomyocytes.
Methods: We present a review of the current literature on the QT interval prolonging effect of macrolides and the role of interactions in their proarrhythmogenic effect based on results of in vitro, in vivo studies and case reports. Our observations were derived from Medline database until June 2008 using the key words „QT interval", „torsades de pointes", „macrolides", „macrolide interactions" and „acquired long QT syndrome". We then reviewed the references of the original articles for additional publications.
Results: The most frequently QT interval prolonging macrolides are erythromycin and clarithromycin. Almost every macrolide-associated QT interval prolongation occurs in patients with multiple risk factors of the following: drug interactions, female gender, advanced age, structural heart disease, genetic predisposition, and electrolyte abnormalities. Macrolides can potentiate cardiotoxicity of some other torsadogenic agents in two ways: by a direct effect on IKr (pharmakodynamic interaction) and by an indirect effect on the metabolisation of the other agents (pharmacokinetic interaction).
Conclusion: We wish to call the health professionals' attention to this potentially lethal side effect of macrolides, and discuss the facilities of prevention and therapy. In conclusion, physicians should avoid prescribing antimicrobials having QT-prolonging potential for patients with multiple risk factors. Recognition and appropriate treatment of TdP are also indispensable.

Microdialysis with Moderately Lipophilic Drugs: What Is Essential to Know for Voriconazole and its Drug Product to be applicable in Pre-/Clinical Microdialysis Settings?

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Introduction: Microdialysis (μD) is regarded as method of choice for the determination of unbound tissue concentrations at target sites. Prior to its in vivo applicability in vitro investigations are crucial. For treatment of severe fungal infections voriconazole (VRC) presents a very potent antifungal. VRC combines several difficult physico-chemical properties for μD application: lipophilicy and low solubility. An i.v. formulation is available containing solubilising sulphobutyl-betacyclodextrine- sodium (SBECD). The aim was to investigate whether unfavourable properties of VRC allow the applicability and conditions of the μD technique in pre- /clinical pharmacokinetic studies.
Methods: A reliable and rapid HPLC assay for the quantification of VRC from small μD volumes was developed and validated. For the in vitro μD investigations a robust and easy-to-handle system was developed permitting physiological conditions. Experiments on the influence of flow rate (0.4-10.0 μL/min), VRC concentration (1.0-50.0 μg/mL), steady state conditions and VRC flow direction regarding the membrane on relative recovery (RR) were performed. In addition, the impact of SBECD in the i.v. VRC formulation was assayed under retrodialysis conditions.
Results: All validation parameters met the criteria set in the international FDA guideline for bioanalytical methods. In vitro μD experiments revealed no binding of VRC to any part of the μD device and therefore VRC concentrations achieved in the dialysate are considered to be sufficient for in vivo investigation. From the observed flow rate dependency a flow rate of 2 μL/min for optimal recoveries for future in vivo investigations was determined yielding adequate sample volumes >12 μL for HPLC assay. RR was independent of VRC concentration. Steady state investigations were performed for the first time and led to recommendations for the in vivo probe calibration solution concentration to range between 100 μg/mL and 200 μg/mL. An impact concerning recovery enhancement of SBECD in the drug product of VRC could be precluded.
Conclusion: The results present the essential prerequisites for further VRC investigations especially for the use of clinically approved Vfend® for determination of relative recovery in pre-/clinical trials.

How Prolonged Exposure to Caffeine Can Affect Dopaminergic Transmission: Evidence from Rats Subchronically Treated with Caffeine and Possible Implications

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Background: Caffeine is a widely consumed psychostimulant, whose effects are mediated by blockade of adenosine A_1 and A_{2A} receptors; an influence of dopaminergic transmission in caffeine-mediated psychostimulation is however acknowledged. The nature of caffeine/dopamine interactions was here investigated by using a rodent experimental paradigm of long-term caffeine exposure.
Methods: Male Sprague-Dawley rats (180-200 g, N = 70) received subchronic-intermittent caffeine (15 mg/kg i.p.) or vehicle (i.p.) every other day for 14 days. Three days after treatment cessation, rats were challenged with either caffeine (15 mg/kg i.p.) or amphetamine (0.5 mg/kg s.c.) to disclose long-term neuroplastic changes. Behavioral evaluation was performed in combination with neurochemical techniques (*in situ* hybridization, microdialysis, binding). Differences between caffeine- and vehicle-treated rats were evaluated by one- or two-ANOVA. Significance was set at $P<0.05$.
Results: Subchronic-intermittent caffeine elicited sensitization to its motor stimulant effects (which reflects the occurrence of neuroplastic changes in dopaminergic transmission) paired with a decrease in the levels of both the mRNA for adenosine A_{2A} receptors (which deeply interact with dopamine receptors) and the mRNA for the early gene *zif-268* (which belongs to the dopamine receptors signaling pathway) after caffeine (15 mg/kg i.p.) challenge. Moreover, caffeine-sensitized rats displayed cross motor sensitization with amphetamine (0.5 mg/kg, s.c.), paired with an increase in the levels of *zif-268* mRNA, and, finally, an elevation in high-affinity dopamine D_2 receptors (D_2^{High}). All the above neurochemical changes were observed in the rats' corpus striatum at three days after subchronic caffeine cessation and were not paired with modifications in dopamine release.
Conclusions: 1) Prolonged caffeine exposure elicits enduring neuroadaptations involving striatal dopaminergic transmission by acting at the post-synaptic level 2) This finding may elucidate the mechanisms underlying the interactions between caffeine and drugs acting on dopamine transmission, including addictive psychostimulants such as amphetamines.

H_2S-induced suspended animation during porcine aortic occlusion-induced ischemia reperfusion injury

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Background: In mice, inhaled hydrogen sulfide (H_2S) produced a "suspended animation"-like metabolic status characterized by hypometabolism and hypothermia, which protected against otherwise lethal hypoxia and hemorrhage[1,2]. The large surface area / mass ratio, however, facilitates cooling in rodents[3], and controversial data were reported on the effects of H_2S in large animals[4]. Therefore we investigated whether H_2S may induce a similar metabolic response and thus be organ-protective during aortic occlusion-induced ischemia/reperfusion (I/R) injury.
Methods: In a first series, 16 pigs undergoing 30 min of aortic occlusion received the i.v. H_2S-donor Na_2S or vehicle started 2 h before ischemia and continued during the whole reperfusion period. During aortic occlusion mean arterial pressure (MAP) was maintained at 80-120% of baseline with esmolol, nitroglycerine and ATP, during reperfusion noradrenaline was titrated to keep MAP at baseline levels. In the second series, 19 pigs underwent prolonged (90 min) aortic occlusion to produce acute renal failure. In both studies the reperfusion period was 8 h.
Results: In the first study Na_2S significantly reduced heart rate and cardiac output without affecting stroke volume, significantly decreased the dose of noradrenaline required to maintain hemodynamic targets, and caused a significant drop in O_2 uptake, CO_2 production, and, consecutively, core temperature. These results were confirmed in the second study. Moreover, Na_2S was reno-protective as demonstrated by a significantly higher creatinine clearance and a lower serum creatinine level at the end of the reperfusion period, which coincided with a reduced oxidative DNA-damage in the kidney tissue as assessed by the tail moment in the alkaline comet-assay.
Conclusions: I.v. Na_2S i) reduces metabolism and energy expenditure in anesthetized large-animals, ii) improves noradrenaline responsiveness during reperfusion after aortic occlusion, and iii) protects the kidney against I/R injury.
References: 1. Blackstone et al, Shock 2007;27:370; 2. Morrison et al, J Trauma 2008;65:183; 3. Leslie, Science 2008;320:155 ; 4. Tisherman & Draben, Pediatr Crit Care Med 2008;9:129
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Neuropeptide FF receptors: a novel target for pain treatment

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Background: morphine and related compounds are well recognized as unsurpassed analgesics for relieving acute and chronic pain. However, their use is limited by the development of tolerance to their analgesic effects that accrues following repeated exposure. It has been proposed that opioid receptor desensitization is at the origin of this phenomenon. A challenging hypothesis is that stimulation of opioid receptors triggers activation of anti-opioid systems that in turns produce hyperalgesia thus diminishing the net analgesic effect of the opioid agonist. This process has been evidenced in vivo both in rats and in man where acute and prolonged opioid treatments induce a long lasting hyperalgesia. Anti-opioid receptor antagonists could therefore represent a promising strategy for opposing to pain hypersensitivity associated with chronic pain especially when an opioid treatment is used. Several neuromodulator systems have been shown to display anti-opioid properties including neuropeptide FF (NPFF) system. However, the lack of pharmacological tools for NPFF receptors has severely limited our comprehension of the in vivo functions of this system.
Methods: in order to identify pharmacological tools for studying the cellular and in vivo functions of NPFF receptor system, we have generated a chemical library of Arg-Phe-NH_2 di-peptide derivatives. This library was screened in competition experiments and a selected compound was evaluated for its ability to prevent the development of opioid-induced hyperalgesia and associated tolerance in rats.
Results: We identified a small potent and selective Neuropeptide FF receptor antagonist, RF9, which can be administered systemically. This compound does not show any effect by itself but can block efficiently the increase in blood pressure and heart rate evoked by Neuropeptide FF thus confirming its antagonist properties in vivo. When chronically co-injected with heroin, RF9 prevents the development of tolerance to opioid analgesia and completely blocks the delayed and long lasting paradoxical opioid induced-hyperalgesia.
Conclusion: Our data indicate that Neuropeptide FF receptors are part of a bona fide anti-opioid system and that selective antagonists of these receptors could represent useful therapeutic agents to avoid the development of secondary hyperalgesia and tolerance induced by opioid treatment.

Multifunctionality of Native and Recombined Proteins of Honeybee Royal Jelly: Assumptions for Application in Pharmacy

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Background: Royal jelly (RJ) is the principal food for the honeybee queen and has been demonstrated to possess several pharmacological activities. The substantial parts of RJ are major proteins, designated as apalbumins (Apa) and belongs to a protein family consisting of nine members with M_r of 49-87 kDa. Because the extreme complexity of protein complement of RJ limits an evaluation of physiological functions of RJ we applied molecular-biological approach to the characterization of Apa and antimicrobial petides with the aims to show if postranslation modification affects production of tumor necrosis factorα (TNFα).
Methods: The native apa1 and apa2 were obtained by ultracentrifugation of RJ at 350 000xg followed by ion exchahge cromatography. The recombinant apalbumins (rApa) and antimicrobial peptides with cluster of 6xHis tag were expressed in *bacterial* or plant hosts and purified by metal affinity chromatography. The level of TNFα produced by murine macrophage (MM) was monitored by ELISA.
Results: Apa1 is oligomeric 420 kDa glycoprotein built up from 55 kDa monomers subunits with ability to create various regular self assembled noncovalent structures similar to those occurring also in RJ. Stimulation MM with the native Apa1, Apa2 and rApa1 (without postranslation modification) reached production TNFα on level in range 900 pg/ml. The oligomeric Apa1 and antifungal and antimicrobial RJ peptide apisimin (5.1 kDa) were less effective as Apa1 in monomeric form. The N-terminal fragment of rApa1 (13.56 kDa) was the most effective elicitor of TNFα release (1414.8 ± 112.2 pg/ml). Other authors showed that C-terminal of Apa1 is precursor of antimicrobial peptides. Our findings indicate that (1) the stimulating effect of Apa1 is derived from its protein domain and it is not affected by postranslation modifications (2) the stimulating effect of honey is based on Apa1, the dominant protein of honey, and (3) Apa1 is regular component of honey and honeybee pollen.
Conclusions. The experimental data on molecular and multifunctional properties of RJ proteins present them as immunomodulators, suppressors of allergic reactions, antibiotic and anti-hypertensive agents, and stimulators of proliferation, and opened real possibility for their application to pharmacy.
Authors' disclosure statement: A honeybee colony as one of the best organized social entities in the nature is armed against pathogens with very efficient exogenous defense system based on multifunctionality of nutritive proteins and antimicrobial peptides. Such a system might become a core of new research program, which would unite activities dispersed till now in various institutions, many of which do not dispose of precisely defined honeybee proteins. We would like to invite pharmaceutical scientific community to collaborate on a new proposed EU research project named as ApiPharm based on (1) purification of native honeybee proteins, (2) preparation of recombinant one biotechnologically by heterologous expression, and (3) determination of their physiological properties in specialized laboratories oriented pharmaco-medically and apidologically. It is a contribution to objective evaluation of therapeutic effects of honeybee proteins before their application on pharmaceutical market.

Surface Modified Nanostructured Carriers of Nevirapine for Brain Targeting

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Background: AIDS is one of the most frightening syndromes worldwide with 5 million new HIV infections a year. Around 14000 people are becoming infected each day & 68 million will die of AIDS BY 2020. HIV potentially harbors in different reservoirs like CD4+T lymphocytes, CNS, lymph nodes, GALT, genital organs, kidney, liver, eye & lungs. Antiretroviral drugs lack target potential to HIV reservoirs. Specific engineering of nanosystems with right drug candidate promote transport across biological barriers. The main objective of present research project was to formulation & evaluation of surface modified nanoparticulate drug delivery systems of Nevirapine, Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) with activity against human immunodeficiency virus type 1 for brain targeting.
Methods: Nanosuspensions of Nevirapine were fabricated by high pressure homogenization technique applying 32 factorial design for intravenous application. Further Surface modification of nanoparticles was done using PEG 300,400,1000, Poloxamer 188, Dextran 60 and bovine serum albumin by covalent conjugation. The developed nanodispersions were characterized for physiochemical parameters & stability. The nanoparticles were sterilized & converted into reconstituable form. Formulations were tested for their anti-HIV potency, cytotoxicity and phagocytic uptake in J744.A1 cell line. The in-vivo tissue distribution was carried out in Wister rats using gamma Scintigraphy.
Result and discussions: Nevirapine nanodispersions were successfully prepared. Homogenization parameters and concentration of surfactant had marked effect on particle size distribution. The nanoparticles with mean particle size of 482 nm and drug release with t50% of 0.22 h and t90% of 3.4h were obtained. AFM confirmed surface modification with increase in particle size. Among cryoprotectants, trehalose gave the desired particle size after reconstitution. Developed nanoparticles crossed BBB within 30 min and remained there up to 8h.
Conclusion: Thus we have successfully developed Nevirapine surface modified nanocarriers for brain targeting

Lipid Nanocarriers for delivery of antimalarial drug Primaquine

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Background: Primaquine(PQ), is the only available antimalarial that acts specifically on the pre-erythrocytic schizonts of plasmodium, which reside and multiply in live and is considered to be a crucial weapon against resurgent vivax malaria. PQ has limited bioavailability because of pre-systemic metabolism and excretion and is characterized by severe tissue toxicity when required in high doses to treat severe cases. Targeting of the drug to the site of action, liver, would possibly reduce the dose and overcome the toxicity problem.
Methods: Lipid nanoemulsion and nanoparticles of PQ were prepared using highly specialized short and medium chain triglycerides with combinations of various hydrophilic and lipophilic surfactants & optimized by applying 3^2 factorial designs. Antimalarial activity was investigated using Peter's four-day suppressive test using swiss albino mice. Parasitic culture of Plasmodium bergheii yoelii was injected intraperitonially to induce malaria. After 4h of infection, the formulations were administered at 4 different dose levels, both by oral and parenteral route. From the 5th day onwards, blood smears were collected and observed for the presence of parasites. Parasitemia(%) and mean survival time(MST) was recorded.Bio-distribution and pharmacokinetic studies were performed in Wistar rats. Lipid nanocarriers were administered orally and intravenously and the animals were sacrificed at the stipulated time intervals and drug content in various organs was determined
Results: The nanocarrier systems showed a narrow particle size distribution with mean particle size ranging between 91-117nm, high drug entrapment and 100% drug release within 6-7 h. Control groups showed invasion & ruptured RBCs with 90% parasitemia by day 10 and no animal survival after day 11. The developed nanocarriers showed 3.5 times better suppression of parasitemia and higher MST at 25% lower dose as compared to conventional formulation. PQ showed improved oral bioavailability and higher levels in the liver after incorporation into lipid nanocarriers. Kidney showed much lower concentration of unmetabolized drug, indicating reduced nephrotoxicity with the developed formulations.
Conclusions: Thus lipid nanocarriers have high potential for the delivery of primaquine to liver with enhanced antimalarial efficacy and reduced toxicity.

Targeting HIV Reservoir & Sanctuary Sites Using Peptide Backbone Polyethylene Glycol (PEG) Nanocarriers

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Background: Despite the wide variety of highly potent anti-HIV drugs that have been developed and brought to the clinic over the years, eradication of HIV infection has not been achieved. We have focused our efforts towards the design and development of PEG-nanocarriers for macrophage targeting. Macrophage targeting represents a key challenge in HIV therapy, since they are not only the primary target of HIV infection, but along with $CD4^+$ T-lymphocytes, they are an important source of HIV persistence. Aim: To develop, characterize and evaluate macrophage targeted peptide-based PEG nanocarriers exploiting the formyl peptide (fMLF) and mannose receptors.
Methods: A modular PEGylated peptide [(acetyl-Cys-β-Ala-β-Ala-Lys)$_n$-PEG$_{5k}$] nanocarrier was designed and developed incorporating two and four copies of fMLFK(fluorescein)C. Two aspects were investigated: fMLF copy number required for optimal binding and optimal PEG size for macrophage uptake. One, two, and four-arm PEG scaffold of molecular weights 5, 10, 20, and 40 kDa were used to conjugate up to four copies of fMLFK(fluorescein)C. Nanocarrriers were characterized using amino acid analysis, MALDI-TOF mass spectrometry, and Size-Exclusion chromatography. Receptor expression was confirmed using RT-PCR and Western Blot analysis. Macrophage-like differentiated human U937 cell-specific binding and cellular uptake studies were performed. Binding, avidity, and uptake were evaluated.
Results: Nanocarrrier uptake was found to be energy dependant and mediated by the fMLF receptor. fMLF copy number was found to influence the binding and uptake behavior. Increasing the number of fMLF moieties from one to two resulted in enhanced uptake of 4 fold, but increasing fMLF copy number to four led only to a modest increase. Molecular size was also found to influence the uptake behavior as increasing the PEG molecular weight from 5 to 20 kDa resulted into an increase in the uptake but further increase to 40 kDa led to decreased uptake.
Conclusions: Receptor-mediated endocytotic uptake of nanocarriers was size dependant with optimal size requirement of ~25 nm or <50 nm. Thus, two copies of fMLF along with a molecular size of 20 kDa PEG appears to be a prerequisite for optimum macrophage targeting.

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The Application of Early Bactericidal Activity (EBA) studies in Assessing Antituberculosis Drugs

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Background: Alternative therapeutic regimens with new faster-acting drugs are needed to stop the spread of tuberculosis (TB). Quantitative and reproducible testing methods are vital to formulate appropriate treatments. Studies of the early bactericidal activity (EBA) were established as an initial step to assess new antituberculosis drugs. It measures the ability of individual antimicrobials to kill active growing bacilli in cavities of patients with pulmonary tuberculosis at the beginning of therapy. Objectives: To retrospectively review the value of EBA studies to determine: 1) whether a drug has activity against bacilli in cavities excreted in sputum. 2) The dose size that is just ineffective and the maximum dose above which no improvement in EBA is found (therapeutic margins).
Methods: A minimum of ten treatment-naïve patients with smear-positive pulmonary TB were randomly allocated to standard daily doses of first line drugs for 2 to 5 days with either: isoniazid (H); rifampicin (R) pyrazinamide (Z); ethambutol (E) and HRZ in combination. Nil groups were included for a maximum of 2 days. EBAs for Ofloxacin (O) given daily and rifapentine (RPE) administered once at the beginning of a 5-day study period are also included. Dose titrations were done on H, R and RPE and therapeutic margins were estimated for H and R. EBA measures the decrease in $\log_{10}$ colony forming units (CFU) of *M. tuberculosis* per ml sputum per day. Sixteen hour sputum collections were homogenised, digested with sputasol, serial dilutions were plated onto selective 7H11 agar plates, incubated at 37 ºC for 3 weeks for CFU counts.
Results: The 0-2 day EBAs (and standard deviation) were: H 6 mg/kg, 0.50–0.64 (0.16–0.30) in 5 studies; R 12 mg/kg, 0.17–0.22 (0.05-0.25) in 3 studies; Z 40 mg/kg, 0.003 (0.04); E 25 mg/kg, 0.25 (0.14); HRZ in combination 0.558 (0.16). Succeeding 2-5 day EBAs were: H 0.06 (0.12); R 0.20-0.30 (0.11-0.18). Ofloxacin had a 0-2 day EBA of 0.39 (0.19) and a 3-5 day EBA of 0.17 (0.20). The 0-2 day EBAs for RPE at 600, 900 and 1200 mg were 0.244 (0.189), 0.364 (0.186) and 0.234 (0.199), respectively and the 2-5 day EBAs 0.193 (0.145) 0.251 (0.116) and 0.241 (0.112), respectively. The therapeutic margins for H was 300/15 = 20 and for R 600/150 ≥ 4.
Conclusion: The EBA procedure requires small numbers of patients and can be carried out at relatively low costs to rapidly determine the therapeutic value, dosage and role of new drugs.

Associative learning induces selective changes in the quantitative distribution of GAT-1, a high-affinity γ-aminobutyric acid transporter, in adult mice barrel cortex

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Background: The increase of functional cortical representation of vibrissae in the mouse brain occurs as a result of classical conditioning where stimulation of selected vibrissae (CS, conditioned stimulus) is coupled with aversive reinforcement (UCS, unconditioned stimulus). The plastic change was demonstrated by labeling with 2-deoxyglucose (2 DG) in layer IV of the barrel cortex. We have also shown that functional reorganization of barrel cortex is accompanied by increased density of small GABAergic cells, GAD67 mRNA, and GAD67-positive puncta in the hollows of barrels of the "trained row". The aim of this study was to determine whether GAT-1, a high affinity, GABA plasma membrane transporter is affected by learning.
Methods: Unbiased optical disector counting was applied to sections from the mouse barrel cortex that had been immunostained using a polyclonal antibody raised against GAT-1 C-terminal sequence with standard avidin-biotin complex (ABC) method. Quantification of numerical density of GAT-1 positive puncta was performed for "trained" (CS+UCS n=5), and control groups: which received only stimulation of vibrissae without the unconditioned stimulus (CS n=5) and naive mice (n=5).
Results: One of the most important findings of the experiment is that learning (CS+UCS) induced increase in numerical density of GAT-1 puncta in hollow barrels of trained row of vibrissae (the experimental side value was on the average 50% higher compare to the control side). In contrast, numerical density of GAT-1 puncta was unchanged in both control groups.
Conclusions: 1) Present data suggested that GAT-1 may be involved in learning-dependent changes in layer IV of the barrel cortex. 2) In any event, these changes in the immunolabeling for GAT-1 in hollow barrels of trained row of vibrissae would cause an increased uptake of GABA. (Supported by MNiSW grant 188).

Potential use of denosumab in the treatment of giant cell tumor of bone

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Background: Giant cell tumor (GCT) of bone, or osteoclastoma, is usually a benign but locally aggressive osteolytic neoplasm in which monocytic macrophage/osteoclast precursor cells and multinucleated osteoclast-like giant cells infiltrate the tumor. While the origin of GCT is unknown, the tumor cells of GCT produce chemoattractants that can attract osteoclasts and monocytic osteoclast precursors, such as receptor activator of NF-KB ligand (RANKL). RANKL is over-expressed in the stromal-like tumor cells of GCT, and is a key mediator of osteoclast formation and survival that could recruit osteoclast-like giant cells to the tumor. The potential role for tumor cell-osteoclast interaction/co-dependence, as in a paracrine loop in which the osteoclast-like cells support the stromal tumor cells, in GCT is unknown. New agents that inhibit osteoclastogenesis via the RANK/RANKL pathway, such as denosumab (a fully human monoclonal antibody against RANKL), may be useful in the treatment of GCT.
Methods: Gene expression in 8 GCT was done by microarray using the Affy U133 chip set. A multi-center open-label phase II trial, of patients with GCT treated with denosumab, 120 mg monthly, with loading doses on days 8 and 15 of month 1 was reviewed. The primary endpoint was tumor response (elimination of ≥90% of giant cells or no radiographic progression of the target lesion) at week 25. In an interim report, 24 patients had received denosumab and 15 were evaluable for efficacy.
Results: Gene expression suggested an osteoclastogenic environment in GCT, with RANKL overexpression. In ongoing trials, 13 of 15 patients had a tumor response: 9 of 9 had a histologic response, and 4 of 6 had a radiographic response. The 2 patients who did not meet radiographic response criteria had stable disease per investigators. Clinical benefit was reported in 9 patients, including reduced pain and increased range of motion. No treatment-related serious adverse events or neutralizing anti-denosumab antibodies were reported.
Conclusions: In the interim analyses reported to date of a multi-center phase II trial, 13/15 evaluable patients with recurrent or unresectable GCT responded to denosumab treatment. The results suggest a possible trophic effect of the recruited osteoclasts on the primary tumor cells. Further investigation of denosumab as a new therapy for GCT is warranted.

Treosulfan-Containing Regimens – an Option to Overcome Transplant-Related Toxicity in Children with Refractory Leukemia

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Background: Allogeneic hematopoietic stem cell transplantation (HSCT) in refractory leukemia patients is associated with high transplant-related morbidity. Treosulfan is a myeloablative alkylating agent that is recently used in conditioning both in children and adults and shows lower non-hematological toxicity, especially comparing to busulfan. We explored treosulfan-containing regimens in children with refractory leukemia.
Methods: 24 patients (17 boys and 7 girls) of median age 7 years (range, 2-17) underwent HSCT from allogeneic donors: 6 matched related, 13 matched unrelated, and 5 haploidentical. All children had refractory leukemia (acute myeloid leukemia – 16, juvenile myelomonocytic leukemia – 3, chronic myeloid leukemia – 1, acute lymphoblastic leukemia – 3, acute biphenotypic leukemia –1), 79% of patients (19/24) didn't achieve any remission to the moment of HSCT. All haploidentical transplants were CD3, CD19 depleted with usage of CliniMACS device. Conditioning regimens consisted of treosulfan 36-42 gr/m^2, fludarabine, antithymocytic globulin ± thiotepa or melphalan. Average number of transplanted cells was 10,2x10^8/kg (range, 1,6-42) for nucleated cells and 6,6x10^6/kg (range, 1-14) for CD34 cells.
Results: All except one patient (96%) achieved stable engraftment at a median of 17 days after transplant. Extramedullary toxicity was rather limited: 16 patients (67%) experienced only mild mucositis. Incidence of acute "graft versus host" disease (GVHD) was 61% (14/23), but only 5 patients presented grade 3-4 acute GVHD. 27% of children (4 of 15 survived to Day 100) developed chronic GVHD (only one case was extensive). 15 of 24 patients died (62,5%) due to relapse in 9 cases (60%), infections in 4 (27%), and acute GVHD in 2 (13%). Two children underwent second HSCT; in 8 children donor lymphocyte infusions were used. 9 of 24 patients (37,5%) survive in complete remission.
Conclusions: In this group of patients with refractory leukemia and unfavorable prognosis without HSCT, treosulfan-based regimens showed reduced regimen-related toxicity, but intensive myeloablative activity and excellent engraftment achievement. Treosulfan could be recommended as a safety regimen compound in pretreated children with refractory leukemia not eligible for standard myeloablation.

Genetic aspects of tramadol PK and PD

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Background: Tramadol, widely used analgesics, is mainly metabolized into an active metabolite O-demethyltramadol (M1) via polymorphic CYP2D6.
Aim of our studies were to objectify PK properties and opioid action of tramadol in subjects with different CYP2D6 and MDR1 genotypes and to evaluate the clinical efficacy of tramadol in acute pain in patients after knee artroscopy.
Methods: Three studies with healthy volunteers (n=87) and one study with patients after knee artroscopy (n=100) were conducted. Volunteers received a standard dose of 100 mg slow release tramadol tablet, while patients were treated by on demand regime after knee surgery. Samples for PK analysis were collected from healthy volunteers over 24 h post-dose and drug – induced miosis over 12 h post dose. Tramadol and M1 analysis were done by GC-MS method. Pupillography was performed using monocular infrared pupillograph „Pupilscan II(TM)" in a quiet and fully darkened room. Visual analogue scale with werbal description, drug consumption, and neccesity to use emergency pain treatment were used to evaluate analgetic efficacy in pateint group.
Results: Tramadol displayed phenotypic pharmacokinetics and it was possible to identify CYP2D6 PM subjects with >99% confidence from single blood sample taken between 2.5 and 24 h post-dose. MDR1 genotype affected PK parameters of tramadol mainly in CYP2D6 PM subjects. Mean (±SD) differences of basal and 8 hours drug-constricted pupillary diameters were 1,36 (±0,68); 0,77 (±0,62); and 0,33 (±0,34) mm in groups of extensive, intermediate, and poor metabolizers, respectively. A tendency towards better analgetic efficacy in CYP2D6 EM patient group was also observed.
Conclusions: Tramadol may be used as a convenient CYP2D6 phenotyping tool and that a pharmacokinetic-pharmacodynamic interaction produces two discernable high and low response phenotypes with respect to PK and pupillary constriction. MDR1 seems to affect PK into a lesser extend than CYP2D6. Genetic predisposition for clinical efficacy need to be confirmed.
Acknowledgement: The author would like to thank Michael Friedrich Bőttcher for Pupillscan II. This study was supported by grant No. MSM 0021620820, MSM0021620849 and GACR 305/08/P069.

Chromosome Damage in Peripheral Lymphocytes of Children with Urinary Tract Infection after Antimicrobial Therapy with Nitroheterocyclic Compounds

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Background: Nitroheterocyclic compounds such as nitrofurantoin [N-(5-nitro-2-furfurylidene)-1-aminohydantoin] and furagin [N-(5-nitro-2-furyl)-allylidene)-1-aminohydantoin] have been used effectively in the therapy of urinary tract infection (UTI) for many years. Since recurrence of UTI is common in susceptible individuals, the objective of the present study was to determine whether nitrofurantoin and furagin, used for long-term low dose antimicrobial prophylaxis of UTI, may induce chromosome aberrations (CAs) and sister chromatid exchanges (SCEs) in lymphocytes of the treated patients.
Methods: Cytogenetic analysis was performed in 153 blood samples from 109 children aged from 0.2 to 13 years and suffering from UTI. The treatment consisted of oral administration of nitrofurantoin or furagin at doses of 5-8 mg/kg/day for the first 7 days and at doses of 1-2 mg/kg/day for the rest of the treatment period. Blood was sampled before the start of the therapy and after 1, 3, 6 and 12 months of the therapy. For each subject, three lymphocyte cultures were set up according to conventional techniques. CAs were scored in 100 first mitotic division cells and SCEs in 25-50 second division cells per individual.
Results: CA frequency was not affected by age, nitrofurantoin or furagin therapy or previous treatment with antibiotics. The only effect on CAs was a higher frequency of CAs (mainly acentrics) due to urethrocystography that preceded the therapy. Analysis of different types of CAs revealed two statistically significant trends: decrease in the frequency of acentrics (Y) with the time elapsed since urethrocystography (X) (Y=1.793-0.062X, r^2=0.77), and increase of chromatid exchanges (Y) with duration of furagin therapy (X) (Y=0.066+0.019X, r^2=0.83). SCE frequency was not affected by age, antibiotic treatment, urethrocystography and nitrofurantoin therapy. A time-independent significant increase in SCE frequency was found in lymphocytes of children treated with furagin.
Conclusions: 1) Furagin induced SCEs and CAs (chromatid exchanges) in lymphocytes of the treated patients. Nitrofurantoin was not genotoxic. 2) Since both nitrofuratoin and furagin are interchangeable in a sense of their antimicrobial efficiency, preferential use of nitrofurantoin in treatment of UTIs might be recommended.

Cancer Immunotherapy with Adenovirus-IFN (TG1042)

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Background. Primary cutaneous lymphomas comprise a heterogeneous group of lymphoproliferative disorders characterized by clonal accumulation of T-lymphocytes (CTCL) or B-lymphocytes (CBCL) homing to the skin. While a variety of registered therapies, including immunotherapies exist for CTCL, no registered therapy is available for CBCL, apart from radiotherapy and surgery. For the management of relapsing CBCL, after first line radiotherapy or surgery, various off-label therapeutic options are being explored. Presently all current treatments including radiotherapy and surgery do not cure the disease but lead to relapses at various frequencies, indicating further medical need for alternative therapeutics. Systemic administration of recombinant IFN- has shown promising response rates in CTCL, however, therapy with recombinant cytokines is limited by their short half-life and significant side effects upon systemic administration. Transgene SA is developing TG1042 (Ad5IFN-) which is based on a replication- deficient adenovirus type 5 (Ad5) carrying the human IFN- gene. TG1042's anti-tumoral activity relies on the anti-proliferative, immunomodulating and anti-angiogenic activities of the transgene-encoded IFN- cytokine.
Methods. The investigational treatment consists of intralesional injections of TG1042 at a dose of 5 x 10^{10} viral particles (vp) per lesion into up to six lesions at days 1, 8, 15 of a monthly treatment cycle. Patients are treated for up to 4 cycles.
Results. An open-label, multicenter, dose-escalation TG1042.01 phase I/II trial enrolled a total of 39 patients with advanced cutaneous T-cell lymphoma (CTCL) and multilesional cutaneous B-cell lymphoma (CBCL). Intralesional injections of TG1042 included a dose escalation regimen in the phase I part. Treatment with TG1042 induced a high rate of local clinical responses: 19 (57%) of 33 evaluable patients responded to the treatment with 9 complete responses (CR) and 10 partial responses (PR). All five enrolled patients with CBCL responded to the TG1042 treatment (100% response). Concerning safety, the results from this phase I/II trial demonstrated that TG1042 was well tolerated up to the highest dose level (3 x 10^{11} vp). Adverse events (AEs) were mostly of grade 1 and grade 2. The most common registered AEs were injection site reactions, fever, chills, and fatigue. A multicenter, open-label TG1042.06 phase II study of intralesional administration of TG1042 in relapsing CBCL patients (PCMZL, PCFCL) is now enrolling patients at centers in Switzerland, France, USA, Serbia, Croatia, and Poland. This is the first clinical trial ever performed in CBCL with a large cohort of 41 patients. Twelve patients are currently enrolled in the study. Interim results of the ongoing phase II study will be presented.
Conclusions. Adenovirus-based cytokine transfer may represent a new treatment modality for cutaneous lymphomas and especially for CBCL given the limited number of treatment options and the lack of registered drugs for this indication. TG1042 may become a convenient treatment option for patients who would prefer other treatments options than radiotherapy or surgery.

The cytostatic treatment effect on nucleoli of leukaemia cells at the single cell level

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Background: Nucleoli are multifunctional organelles. Their structure, cytochemistry and size reflect main cell states as proliferation, differentiation, resting state, ageing and death.
Methods: Nucleoli and main nucleolar components of leukaemia cells untreated or treated with various cytostatics were visualised by light microscopic cytochemistry for demonstration of DNA, RNA and silver stained. nucleolar organisers (AgNORs). Electron microscopy visualised main nucleolar structural components, i.e. chromatin, RNA containing structures and Fibrillar Centers (FCs) corresponding to AgNORs.
Results: Proliferating less differentiated cells are characterised by „active" RNA transcribing large nucleoli containing multiple AgNORs and FCs. However, large nucleoli were also detected in leukaemia myeloblasts after cytostatic therapy with histone deacetylase inhibitors or laevulinic acid induced photodynamic treatment. In such nucleoli AgNORs were markedly reduced and electron microscopy demonstrated the translocation of FCs to the nucleolar periphery followed by their expulsion from the nucleolus.
Ring shaped nucleoli are characterised by a single AgNOR surrounded by a RNA containing peripheral shell. They are in „resting" cells and reflect the reversible decrease of RNA transcription. They may be produced from „active" nucleoli by intercalators or imatinib. The presence of „active" and „resting" nucleoli in one cell was noted in leukaemia cells resistant to the cytostatic treatment with antimetabolites, alkylating or intercalating drugs, and histone deacetylase inhibitors. The photodynamic treatment also produced that phenomenon.
Micronucleoli (nucleoli smaller than 1μ) with a characteristic structure accompanying terminal differentiating and apoptotic state are produced by all types of current cytostatics. However, the effect of cytostatics may differ. In patients with chronic myeloid leukaemia hydroxyurea produced a larger number of myeloblasts with micronucleoli than interferon.
Conclusions: The fine structure and cytochemistry might provide useful information on the effect of cytostatics on leukaemia cells at the single cell level with a full respect to cell differentiation and maturation stage.
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Pharmacokinetics and Biological Effects of the Recombinant Human Bone Morphogenetic Protein-7 in Vivo

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BACKGROUND: The cellular and molecular mechanisms that determine autonomous hematopoietic competence in the Bone Morphogenetic Protein induced osteoblastogenesis and angiogenesis have not been characterized yet. AIMS: In the present study we investigated biological activity's of recombinant human Bone Morphogenetic Protein-7 (rhBMP-7) during osteogenesis and their effect on the autonomous hematopoetic system.
MATERIAL AND METHOD: On 14 New Zeealand White Rabbits surgicaly were created full-thickness non-critical size mandible defects. On 7 animals defects were treated with 100 micrograms of the rhBMP-7 in collagen as carrier (ACS) and on other group of 7, defects were treated with autologues iliac crest bone grafts (control). Markers of the osteogenesis function included alkaline phosphates enzyme (ALP) specific activity. Histology analysis were performed on day 7,14,30 and 60 postoperative and C-T scan with Bone Mineral Density analysis done on day 30 postoperative. Standard CBS tests from the periphery blood done preoperative and day 5,14,21,30 postoperative.
RESULT: Day 7, histology analysis rhBMP-7 induced new form tissue showed granulation tissue and strong angiogenetic response and day 30 mesenchymal tissue enrichement with osteoblast and plenty of new formed capilars and vessels network. Day 60 mature bone formation with collagen and osteocytes was detected. 5 of total 7 test animals showed completely bridged defect with new formed bone. Osteogenesis was characteristic for intramembraneous ossification, without cartilage involvement. ALP activity was significantly increased on day 21 on test group compared with controul group detected day 30. C-T and Bone Mineral Density showed average density of the new formed tissue between 413 mg/cm3 and 519 mg/cm3, determinate by program as the bone, on all animals. All hematologist values were in physiologic range on both groups.
CONCLUSIONS: 1. rhBMP-7/ACS constuct showed stronger osteoinductive capacity then autologue bone graft, gold standard in conventional medicine; 2. Osteoinductive effect rhBMP-7 during osteogenesis, didn't affect hematopoetic competence determinate by the Er. Le, HCR and Hg; 3. Strong BMP induced osteogenesis effect is prerequisite et least by strong angiogenetic response.

[C11]Mirtazapine: Magic Bullet for PET Brain Imaging of Antidepressants

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Background: Dr. Paul Ehrlich knew the importance of a close match between spatial features of drugs and biomolecules, and his vision continues to guide research in medicinal chemistry. Today, positron emission tomography (PET) is often used during drug development to explore the inner workings of the body.
Methods: Over the years, we have used PET to explore actions of antidepressant drug in the brain in an effort to discover "magic bullets" for assessing neuroreceptors that may mediate causes and cures of psychic depression.
Results: Our research has focused in recent years on the antidepressant compound mirtazapine, radiolabelled with C-11 for PET. We have found that the PET radiotracer has suitable properties for neuroimaging of antidepressant binding sites in the brain of laboratory animals and humans. The receptor occupancy obtained by therapeutic doses of mirtazapine can be quantified by [C11]mirtazapine in regions of the living human brain. Studies of the enantiomers of [C11]mirtazapine in vivo and in vitro showed differences in the stereoselectivity of the findings.
Conclusion: [C11]Mirtazapine provides a reliable PET-procedure for quantifying receptor sites involved in antidepressant actions.

Resolution of cancer and infection induced wounding is an essential factor in immune enhancement. The answers to why and how revealed

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Background: The lifecycle of Cancer can be simplified into two phases of the disease; the earlier phase where abnormal growth is a prerequisite for carcinogenesis and the later phase, malignancy, where abnormal growth cannot continue without harnessing the body's wound response. Induced wounding in the stroma provides many benefits to Cancer progression including destruction and remodeling of competing healthy tissue, growth of new blood vessels and immune suppression.
Methods: Systems Analysis of published journal literature
Results: Evidence shows that Cancer induced wounding is critically dependent upon up-regulation of the Angiotensin II type 1 (AT1R) receptor. Classically associated with the vascular system, expression of AT1R is a systemic cellular response to oxidative, hypoxic and physical stresses. Activation of AT1R by Angiotensin II leads to the production of a host of pro-inflammatory mediators including cytokines, chemokines, adhesion molecules and other factors such as TGF-beta, VEGF and Matrix Metalloproteinases. Early clinical studies utilising Angiotensin Receptor Blockade in Cancer and other chronic inflammatory diseases (many of which having, or suspected to have, an infectious component) demonstrate increased patient survival, reduced biological disfunction and pain.
Conclusions: Lessons learnt from a systems analysis of the behaviour of Angiotensin in Cancer explains that the induced 'Wound that never heals' is an environment that is of vital importance in suppressing the adaptive immune response and that Cancers and other infections utilise chronic inflammation in order to spread and evade learned immune responses. Angiotensin Receptor blockers (ARBs) have a safety profile, with side effects comparable with placebo even at supramaximal dosage. These drugs can be used alone, turning malignant tumours benign, however ARBs would be synergistic with conventional and developing therapies, especially vaccines, greatly improving effectiveness.
Authors' disclosure statement (not counting towards the character count):
Gary R Smith is a founding director of Perses Biosystems Ltd. The goals of the company are to drive laboratory and clinical research into the role of angiotensin receptors in disease management. Although we envisage these activities to be humanitarian (non-profit making) in nature, our long-term ambition is to identify additional drug targets and agents that could work in combination with ARBs to treat most diseases.

Finding a New Vaccine in a Ricin Protein Fold

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Background: Early attempts to develop a subunit vaccine against ricin were impeded by safety concerns arising from residual toxicity and the unwanted aggregation or precipitation caused by exposure of hydrophobic surfaces on the ricin A-chain (RTA) in the absence of its natural B-chain component. A structure-based solution to the problem was undertaken to arrive at an optimized protein formulation that shows greater resistance to thermal denaturation, increased solubility and stability, and elimination of catalytic (N-glycosidase) activity.
Methods: A structure-structure alignment between ricin A-chain and pokeweed antiviral protein (PAP) was performed to determine the relative hydrophobicity of the carboxyl terminal regions. Computational analysis of the structures provided insight into the hydrophobic effect causing the ricin A-chain subunit to aggregate and precipitate. C-terminal ricin A-chain truncation constructs were expressed in E. coli and purified by using conventional chromatography. Mice and non-human primates (NHP) were used to evaluate the efficacy of this vaccine.
Results: This comparative analysis between RTA and PAP pointed to a partitioning of function between the two domains of RTA, with the N-terminal sequence of RTA optimized to serve as an "anchoring" fold. In this model, the C-terminal RTA domain is a later functional modification that contributes to ribosome-inactivating protein (RIP) activity, and in two-chain RIP also provides the hydrophobic interfacial region with the lectin subunit. We exploited this structural hierarchy of ricin by splitting the functions to eliminate the undesirable hydrophobic surface of the C-terminus, while preserving the integrity of the B-cell and T-cell epitopes. Computer models of the RTA-RNA assembly led to removing residues 199-267 to obtain truncated RTA 1-198. Further modeling of solvent effects for RTA 1-198 led to the removal of a loop region (RTA 34-43) that unfavorably increased overall solvent accessibility of the protein. The creation of the polypeptide, rRTA 1-33/44-198 (RVEc), further optimized the compactness of the structure, thereby disfavoring protein unfolding and aggregation. The RVEc vaccine showed no detectable RIP activity, thermal stability greater than its parent RTA molecule, no detectable precipitation unlike previous candidates, no detectable vascular leak activity in an in vitro cell assay, and protected vaccinated mice and NHP against lethal challenge with aerosolized ricin
Conclusion(s): Protein engineering can be used to increase stability and solubility of recombinant subunit proteins enabling increase soluble expression from heterologous host systems and higher production yields during manufacturing while maintaining antigenic properties and eliminating undesirable features.

Rapid intervention: the role of antivirals in containing or controlling pandemic influenza

SMITH JR

International Medical Leader: Tamiflu, F. Hoffmann-La Roche Ltd, Basel, Switzerland
[Roche: EHRLICH II character count (not including spaces): 2,200; current character count: 2,198]

The continuing spread of the influenza A(H5N1) virus among poultry in Southeast Asia, Europe, the Middle East and Africa represents the most serious risk of a human influenza pandemic in decades. As no matched vaccines, and only limited amounts of pre-pandemic vaccines, will be available at the start of a pandemic, the principal interventions are likely to be antivirals and social distancing. Modelling data suggest that antiviral treatment of infected individuals and post-exposure prophylaxis (PEP) of household contacts, combined with containment, could halve infection rates during a pandemic.
The oral antiviral agent oseltamivir (Tamiflu®) is active against multiple influenza strains and is the only neuraminidase inhibitor to have shown activity in the clinical management of patients infected with H5N1. As such, oseltamivir is the only antiviral that is strongly recommended by WHO for the treatment of individuals with confirmed or strongly suspected H5N1 infections and PEP of high-risk exposure groups. WHO has recently highlighted that 'early treatment with oseltamivir is recommended, and data from uncontrolled clinical trials suggest that it improves survival', underscoring the need for rapid medical intervention.
WHO advises stockpiling of oseltamivir to ensure that sufficient supplies are available for pandemic use, as timelines for 'surge production' will not meet the requirements for a rapid response. Roche, the manufacturer of oseltamivir, has scaled up production capacity and can now supply up to 400 million treatment courses annually. At present, 85 governments worldwide have oseltamivir stockpiles. Cumulatively, this is sufficient to treat <5% of the world's population. To maximise efficacy, individuals infected with the pandemic virus should receive treatment as soon as possible (ideally within 2 days) after symptom onset. If this cannot be achieved, stockpiles may not be used to maximum benefit.
Roche is holding a global stockpile of 3 million treatment courses as a 'rapid response stockpile' to be donated for use by WHO exclusively at the site of outbreak of a pandemic in an attempt to contain or slow its spread. Roche has also donated a regional stockpile consisting of a further 2 million treatment courses to WHO to serve the needs of developing countries. Once a pandemic is declared, Roche will fill oseltamivir orders as follows: 1) delivery of rapid response stockpile to WHO; 2) fulfilment of existing pandemic orders from governments and other groups; 3) increase in containment effort with WHO and other international agencies.

Activation of Ancient Stress-resistance Pathways by Molecular Triggers, Age and Disease Intervention

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Background: Not only the genetic blueprint, but also the ability to access survival pathways by epigenetic signaling determine species survival. The pathways, though present and thought lost to man, only require an activation trigger of latent protective mechanisms. Molecular mimics of the environmental cues have emerged as triggers of the protective and rejuvenating molecular pathways from the studies from this laboratory and from numerous other investigators. Hibernation is the environmental response to stress. Delta opioids are used to tolerate hemorragic stress in our pubished studies.
Objectives:To assess the effect of one such agent to tolerate ischemic stress of hemorrhage, deltophin D, (DeltDvar) a mimic of hibernation factor, our following experiments were preformed.
Methods: Rats were fitted with femoral arterial and venous catheters for measurements of mean arterial pressure (MAP), heart rate (HR), and intravenous (i.v.) injections of isotonic saline, 1 mg/kg Delt-Dvar, or 2 mg/kg Delt-Dvar. During hemorrhaging, 30% (5 mL) of total blood volume was collected from the arterial catheter. MAP-HR was fitted to a logistic equation to determine baroreceptor reflex properties.
Results: Saline and 1 mg/kg Delt-Dvar rats treated posthemorrhage had similar MAP and HR after hemorrhage. In contrast, 2 mg/kg Delt-Dvar administered after hemorrhaging led to a faster and more complete recovery of MAP than compared with the other groups. In hemorrhaged rats, the average HR gain (bpm/mmHg) after 2 mg/kg Delt-Dvar treatment was greater and the BP50 (BP at one-half the HR range) was significantly lower than after saline treatment.
Conclusion: After hemorrhage, 1) stimulation of Delt Dvar opioid receptors leads to improved MAP, and this recovery may involve a change in baroreflex sensitivity. 2) In our other studies, using, DeltE, another peptide, significant extension of survival without fluid resuscitation was found. Since deltorphins preserve function during stress, and strength muscles during hibernation, agonists of the delta opioid receptor may stimulate type 2 muscles in the absence of exercise. The longevity pathways to be activated by pharmaceutical triggers are identified by their conservation through evolution to withstand stress. The magic bullet(s) would include all of the mimics of environmental cues or selected mimics when the activated pathway would intervene in specific diseases.

Novel Modular Nanotransporters which Significantly Enhance Efficacy of Transported Drugs and Impart Cell Specificity to Them

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Background: A major challenge in the development of specific and effective cancer treatments is the fact that exploiting a molecular target that is accessible (e.g. cell membrane or extracellular matrix) is critical for achieving tumor selectivity while delivery of the therapeutic to the cell nucleus is generally required for maximizing the therapeutic effect. An intriguing approach to this conundrum is to utilize a hybrid molecule to achieve both goals by linking together modules with different functionalities.
Methods: We have employed recombinant technology to develop targeted therapeutics that include modules for addressed delivery both to tumor cells and into compartments within these cells that are the most sensitive to the drug. Our modular nanotransporters (NT) are polypeptides possessing a) an internalizable ligand module providing for target cell recognition and subsequent receptor-mediated endocytocis of the NT by the cell; b) an endosomolytic module ensuring escape of the NT from the endosomes; c) a module containing a nuclear localization sequence, thereby enabling translocation of the NT into the cell nucleus; and d) a carrier molecule for attachment of a drug.
Results: We produced different NTs containing different ligand modules enabling recognition and internalization of the NTs by the following target cancer cells: melanoma, glioma, epidermoid carcinoma, acute myeloid leukemia and neuroblastoma. Cytotoxic efficacy of either □-emitting radionuclides or photosensitizers transported by the NTs turned out to be 10-3000 times higher than that of free drugs. Moreover, the NTs impart cell specificity to the drugs: e.g., free photosensitizers are equally photocytotoxic for target and non-target cells, whereas if they are attached to the NT they become not photocytotoxic for non-target cells at the concentrations that were photocytotoxic for target cancer cells.
Conclusions: Cell specificity and high efficacy of many therapeutics can be achieved with the use of modular NTs with preset properties, which would ensure recognition of the desired target cell and subsequent directed transport to the subcellular compartment of choice.

Predatory Bdellovibrio Bacteria as Living Antibiotics-Nature's Magic Bullets to Treat Antibiotic-Resistant Gram-Negative Infections

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Background: Bdellovibrio bacteriovorus is a naturally predatory bacterium that attaches to other Gram-negative bacteria invading the periplasm. Bdellovibrio cells are small and highly motile due to a single sheathed flagellum and efficiently collide with their prey in liquid media and in biofilms. Once they have entered the prey bacterial periplasm they become sessile and attach to the inner membrane of the prey, killing it and then they release organized waves of enzymes which hydrolyse the prey cytoplasm, allowing uptake of prey nutrients into the Bdellovibrio which is living in the periplasm. The Bdellovibrio grow as a long sausage-shaped cell until the prey nutrients are exhausted, then they septate into small cells, regain flagellar motility and burst from the husk of the exhausted prey to invade and kill more bacteria. This is a fascinating life-cycle but what is more important is that prey bacteria for Bdellovibrio include Proteus, Escherichia, Salmonella, Serratia, Acinetobacter, Pseudomonas, Burkholderia and many other important Gram-negative pathogens of man and animals. We are working to investigate the natural predatory properties of Bdellovibrio and to apply them to treat infections where antibiotic resistance is an emerging problem.
Methods and Results:. We collaborated in the sequencing of the first, and to date, only published Bdellovibrio genome in 2004 and since then have been examining, using chip arrays the transcription from that genome when the Bdellovibrio attack prey bacteria. We have verified our results using QPCR and have then inactivated key genes that we found to be upregulated to see the effect upon predation. We see the genes and their products that we have studied so far as the "first bite" of the Bdellovibrio into prey and are beginning to understand the order in which prey are degraded. We have also established that Bdellovibrio preys efficiently upon bacteria in serum and that typeIV pili are important for its entry into prey
Conclusions: Bdellovibrio is now being converted from a charming microbiological curiosity, to a potent curative for the next wave of human and animal infections, using our combination of genetic and microscopic approaches.

LC-MS/MS Shotgun Proteomics of Lung Cancer Pleural Effusions Identifies the Prognostically Relevant Epithelial-to-Mesenchymal Transition Protein Periostin

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Background: Malignant pleural effusion of advanced lung cancer is a valid source for detection of secreted N-glycosylated biomarker proteins (N-GP), because tumor cells grow during weeks in this liquid. Therefore, such N-GP effusion profiles are likely to contain tumor progression factors. Among them, epithelial-to-mesenchymal transition (EMT) proteins are of particular interest, since EMT is a key program facilitating invasive tumor growth into its surrounding desmoplastic stroma.
Methods: Malignant pleural effusions of 5 patients with lung adenocarcinoma and 5 non-malignant controls were used for triplicate N-GP capturing by solid-phase extraction. After trypsin digest and PNGase F release, liquid chromatography followed by tandem mass spectrometry (LC-MS/MS) was performed. For biomarker protein validation, a tumor tissue microarray of 533 patients with surgically resected non-small cell lung cancer (NSCLC) was analysed by immunohistochemistry.
Results: In the total of 10 effusion samples, 170 non-redundant proteins were detected with probability 0.9 to 1. The specificity for the N-glycomotif was 88%. Mass spectrometric penetration into the moderate to low protein concentration range (mikro-nanogram/mL) occurred. The EMT protein periostin was confidently identified in several malignant effusions. Of the 533 NSCLC patients, 48% had squamous cell carcinoma, 47% adenocarcinoma and 5% adeno-squamous carcinoma. High protein expression of periostin in either peritumoral desmoplastic stroma or tumor epithelia, independently scored by two pathologists, correlated with male gender, higher stage, higher pT category and larger tumor size; and in only stroma with tumor relapse (all p-values <0.05). Further, high stromal expression was found to be a prognostic factor for decreased progression-free survival on univariate analysis (p-value 0.007).
Conclusions: Pleural effusion is a useful biomarker source for lung cancer. Reduction of sample complexity by N-GP capturing allows detection of low abundance biomarker proteins. The EMT protein periostin is closely associated with advanced disease and may thus be integrated in progression models of NSCLC.

Treatment of Agitation in Dementia: Polypharmacy for symptom relief as the Magic Bullet

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Background: Few controlled studies are available to guide the clinician in treating potentially assaultive elderly individuals with psychiatric disorders. This is an important concern, with up to 90% of dementia patients suffering from agitation during its course. Safety concerns limit the use of benzodiazepines and antipsychotic medications, making anticonvulsants an attractive alternative. With no one medication as a magic bullet, the side effects profile of a given medication may be used to its advantage in treating a patient's unique target symptoms. In other cases, targeted dosing of several medications concomitantly for specific symptom relief may yield the most effective results. Each medication has side effects that may be contraindicated in this population.
Methods: We reviewed the current research on the efficacy, safety and tolerability of anticonvulsant medications used for individuals over 60 with agitation due to dementia. Recommendations for use under specific circumstances are made, depending on the specific symptoms of the patient.
Results: Gabapentin, useful in patients with co-existing pain, may cause ataxia. Oxcarbazepine, with few efficacy studies, is associated with severe hyponatremia. Carbamazepine, important in the treatment of bipolar disorder and pain disorders, may cause hyponatremia and pancytopenia. Topiramate may be helpful in the weight gain commonly induced by other medications such as the commonly prescribed antipsychotic drugs, but is associated with significant cognitive impairment. Valproate, also used for pain syndromes may cause transaminase elevation, pancreatitis, and hyponatremia.
Conclusions: With no medication approved for the treatment of agitation associated with dementia, the clinician has a dilemma in nonetheless needing to treat the out of control yet frail patient. There is no one class of medication that is most effective or best tolerated for this vulnerable population. Better outcomes often are achieved by combining different medications to optimize efficacy while limiting adverse effects. Anticonvulsants are an example of a medication class that may be used to advantage in this way; either to augment the effect of other drugs for therapeutic effects, or to diminish side effects.

Alpha 1 Antitrypsin (AAT) for the Treatment of Autoimmune Diseases

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Background: Autoimmune diseases including type 1 diabetes (T1D) and rheumatoid arthritis (AR) result in self destructions and tissue injuries. Although multiple factors contribute to these diseases, an imbalance of the immune regulatory pathways plays an important role in the development. Therefore, immunoregulatory and anti-inflammatory strategies hold great potential for the prevention of these autoimmune diseases. Alpha 1 antitrypsin (AAT) is a serine proteinase inhibitor and exhibits various anti-inflammatory effects and protects against tissue damage or injury. The goal of our studies is to develop AAT thereapies for the treatment of autoimmune diseases.
Methods: We have tested the feasibility of AAT protein and gene therapy for the prevention of type 1 diabetes in none-obese diabetic (NOD) mouse model and the prevention of RA in collagen induced arthritis (CIA) mouse model. For AAT gene delivery, we used recombinant adeno-associated virus vectors (rAAV).
Results: AAT gene therapy, contrary to control vector (expressing elafin) and saline, decreased insulitis, reduced the levels of autoantibody and prevented type 1 diabetes efficiently and in dose-dependent manner. T cell receptor spectratyping indicated that AAT gene therapy altered T cell repertoire diversity in splenocytes from NOD mice. Adoptive transfer experiments demonstrated that AAT gene therapy attenuated cellular immunity associated with beta cell destruction. Our results showed that AAT protected islet cells against apoptosis and block cytotoxicity of NK cells. Our recent studies also showed that AAT protein and gene therapies significantly delayed arthritis development in CIA mouse model.
Conclusion: Alpha 1 antitrypsin (AAT) has great potential for the treatment of type 1 diabetes and rheumatoid arthritis.

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Drug Resistance in Surgical Infection

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Despite development of new antibiotics and protective surgical techniques, infection remains a serious problem in severely injured surgical trauma patients. Infection is the leading cause of late death in severely injured patients. Although the bacteria responsible for the infections, usually gram negative enterobacteriacae, may be sensitive to antibiotics in vitro, the severely injured patients often do not respond to antibiotic therapy. Evidence suggests that trauma-induced deficiencies in the immune response, particularly involving antigen presentation and cytokine production, interfere with the ability of the patient to clear the infection even after antibiotic treatment. Treatments to enhance the immune response, such as interleukin-2 and interferon-gamma therapy, have been used to enhance the immune response of severely injured trauma patients. Although these treatments have been very effective in animal models, the fact that they are regulatory cytokines and have other effects beyond restoring immune response has made their use in human systems problematic. Active Hexose Correlated Compound (AHCC) is an extract from the mycelia of the Basidiomycete mushroom that is non-toxic and has been shown to enhance immune responses and resistance to gram-negative enterobacteriacae. The use of AHCC in the intramuscular thigh infection of acutely food –deprived mice with Klebsiella pneumoniae, a model of trauma and infection, resulted in reduced bacterial load and total clearance of bacteria over time, which did not occur in controls. Cytokine production was also enhanced in these mice. These results suggest that AHCC treatment may be a novel approach for overcoming "drug resistance" related to an impaired immune response in severe trauma victims. Funded in part by a grant from the Amino Up Chemical Company, Sapporo, Japan.

Targeted Pulmonary Delivery of Aerosolized PGE1: A "Magic Bullet" for Neonatal Pulmonary Hypertension?

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Background: Extensive experience with inhaled nitric oxide in neonatal persistent pulmonary hypertension (PPHN) shows lack of improvement in 30-40% of patients. Inhaled PGE1 (IPGE1) is a potential selective pulmonary vasodilator. Our aim is to characterize the stability, emitted dose (ED) and the aerodynamic particle size distribution (APSD) of PGE1 aerosol generated by the MiniHeart jet nebulizer. An additional goal is to evaluate pulmonary drug delivery and toxicity.
Methods: Chemical stability and ED of PGE1 solution were evaluated during jet nebulization in a neonatal conventional (CMV) and high frequency (HFV) ventilator circuit by High Performance Liquid Chromatography - Mass Spectrometry. A six-stage cascade impactor was used to determine APSD. Pulmonary deposition was assessed with MRI in seven ventilated piglets using T1 weighted spin-echo sequence. Toxicity was evaluated in ten ventilated piglets receiving either high dose IPGE1 or nebulized saline continuously for 24 hours.
Results: There was no significant degradation of PGE1 following nebulization. The ED of PGE1 was 32-40% during CMV and 0.1% during HFV. The PGE1 aerosol had a mass median aerodynamic diameter (MMAD) of 1.4 µm and geometric standard deviation of 2.9 with 90% of particles being < 4.0 µm in size. On MRI, a significant increase in signal intensity (SI) was observed in the lungs 10 min after start of aerosol. At the end of 90 min, the SI increased by 98%. There was no evidence of adverse cardio-respiratory effects related to IPGE1 in ventilated piglets. Histomorphological changes included moderate to severe focal ulceration, flattening of the bronchial epithelium and loss of cilia in the trachea and bronchi.
Conclusions: 1) Nebulization of PGE1 during neonatal CMV or HFV is efficient and results in rapid nebulization without altering the chemical structure. 2) On the basis of the low MMAD, and large proportion (90%) of particles <4.0µm, one can predict predominantly alveolar deposition. 3) This was validated on MRI, with evidence of effective pulmonary aerosol delivery within 10 minutes of contrast nebulization. 4) Inhalation of high dose IPGE1 was not associated with adverse cardiorespiratory effects and produced minimal signs of pulmonary toxicity even after 24 hours. 5) Thus, IPGE1 may be a safe and effective selective pulmonary vasodilator in PPHN.

Oxidative stress in periodontal diseases and associated neoplasias: a role for antioxidants?

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Background: Studies indicate that plaque-associated inflammatory loading in periodontal diseases leading to tooth loss may have associations with developing cancer. There is documentation of a significant association between cancer of the lung, kidney, pancreas, haematological cancers and periodontal diseases. The development of oxidative stress with malignant progression of a tumour has been reported, with evidence of efficacy of antioxidants as anticancer agents. For example, dehydroascorbic acid reacts with homocysteine thiolactone found in cancer cells resulting in the formation of the toxic compound 3-mercaptopropionaldehyde which kills cancer cells. Other effective agents reported in this context are green tea polyphenols, melatonin and vitamin D also reported to be beneficial in periodontal disease outcome. Green tea polyphenols are able to induce apoptosis in various tumour cell systems. This apoptotic mechanism has been shown to be targeted at mitochondria and executed by caspase 3. This has been demonstrated by subjecting tumour cells which had either a caspase 3 deletion or expression of wild type caspase 3 to increasing concentrations of green tea polyphenols indicating mitochondrial targeting. The development of oxidative stress with malignant progression of a tumour has been reported. Drug resistant cell populations can emerge in response to a milieu of oxidative stress. Cellular adaptation is likely to be multifactorial, coordinating factors that induce hypoxia, nuclear factor kappaB (NF-kappaB) and their targets downstream that are linked to resistance mechanisms. This resistance can be overcome by treating the cells with NO mimetic agents to restore their sensitivity to cytotoxic agents both in vivo and in vitro. Mechanisms involved include vascular changes, tumour oxygenation and antioxidant effects, down regulation of the glutathione detoxification / redox buffering system, inhibition of key transcription factors and DNA repair systems.
Conclusions: It is relevant that periodontal diseases are associated with a small but significant overall cancer risk which persists in non-smokers. Periodontal disease may be a useful marker of a susceptible immune system, or directly affect cancer risk as a result of inflammatory loading. Formulation of effective therapeutic dosing of agents shown to have efficacy in this context is a challenge.

Betulonic acid and its alanine amide derivatives - a new multy-target agents for tumor chemotherapy

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Background: A multy-target agents based on natural pentacyclic triterpenoid platforms present a new approach to drug design. They may be use to both prevention and therapy of cancer as well as to be synergistic with standard anti-cancer treatments. Aims: 1) To development a multy-target agents using betulonic acid (BA) as new triterpenoid platform. 2) To study in vivo theirs anti-oxidant, anti-inflammatory, antitumor and antimetastasis activities. 3) To study the effect of agents on potency and tissues damage of standard chemotherapy.
Methods: This study included 80 male mice CBA, 220 female mice C57Bl/6, 160 outbreed male mice. Transplantable tumors were Lewis lung carcinoma (LLC) and mice RLS lymphoma. Agents were administered orally at single (250, 500 mg/kg) or daily (50 mg/kg/day during 7 or 14 days) doses. A standard poly-chemotherapy ACOP (adriamycin, cyclophosfamide, oncovin, prednisolone) was carried out 24 hours before dosing. It was estimated: tumor growth, volume areas of metastasis, dystrophic and necrotic changes in some tissues (morphometry using light microscopy), a thiobarbituric acid-reactive substances in serum, anti-inflammatory activity (histamine- and carrageenane-induced mice paw edema models).
Results: It was synthesized 4 derivatives BA with *d,l*-α-ala or β-ala substitution at C-28 and their methyl esters. All agents at single dose reduce tumor growth (by 10-20%) and inhibit metastasis CLL (by 40-50% as to control). All agents possess anti-oxidant (40-60%) and anti-inflammatory (30%) activities (P<0,05). After 7 days of dosing (50 mg/kg/day) only two acid derivatives have antitumor activity (12-17%), one with β-ala significantly decreases LLC metastasis in lung (by 9 times, P<0,05) and RLS metastasis in kidneys (by 1,7 times). Treatment of mice with this agents after ACOP leads to rise its antitumor (to 1,3 times, P<0,05) and antimetastasis (up to 2 times, P<0,05) potency. The acid derivative with β-ala decreases a necrosis (to 42% as to ACOP, P<0,05) and increases a dystrophic changes (to 28%) in the liver and kidneys. The agent induces a glycogen synthesis in the liver.
Conclusions: Among alanine amide derivatives BA it is found an agent that has high antimetastasis activity, rises a poly-chemotherapy potency and decreases tissues damage.

Lithium and vestibular function in Bipolar Disorder

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Background: Bipolar disorders characterizes by mood changes between mania and depression. Despite lithium is a useful drug for stabilizing mood, the mechanism underlying it is still unknown.
Methods: We studied 2 bipolar I disorder patients during successive depressive and maniac states using electronystagmographic registers after vestibular rotatory stimulation. Slow phase velocity(SPV) of nystagmus was calculated for quantify right and left vestibular activity. Right/Left SPV ratio was assessed for appreciating vestibular lateralization differences between depression and mania. The actual treatment with lithium was inquired in all the mood states.
Results: We recorded 4 different episodes of depression and 4 of mania. In depression we found a 41% diminution of right vestibular activity, and 17% elevation of left. In Mania we found 35.2% diminution of right vestibular activity and 37% diminution of left . Right/Left SPV ratio mean in depression was 0.7±0.1 (mean +SD, 4 records), significantly lower than 1.4±0.1 found in maniac phases (4 records) (p< 0.05, two tailed, Mann-Whitney test). We observed that in 2 cases the vestibular pattern characteristic of maniac state was preceded by the suspention of lithium treatment, and in 2 cases it was reversed after the reposition of the treatment.
Conclusions: We found fluctuations of vestibular dysfunction depending on mood state in Bipolar Disorder patients. Left vestibular activity was the parameter that varied the most between mood states, for this reason we suggest that the stabilizing efect of lithium depends on the capacity for modulating left vestibular nuclei directly or through the modulation of left side vestibular nuclei aferents.

Metalloporphyrins are Versatile and Powerful Therapeutics: Biomimetics of SOD, Peroxyredoxin, and cyt P450

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Background: Mn-porphyrins with proper substituents possess high superoxide and peroxynitrite scavenging activity, and can modulate redox-based cellular signaling pathways thereby exerting protection in radiation, cancer, diabetes, Alzheimer's, stroke, cerebral palsy etc. Fe- more than Mn- analogues are efficacious cyt P450 mimics, while Zn-porphyrins are potent photosensitizers.
Mimicing cyt P450
Methods: We compared different Fe- and Mn-porphyrins in their efficacy to catalyze hydroxylation (activation) of cyclophosphamide (CP) to 4-OH-CP, the active anticancer metabolite, under biologically relevant conditions. Thus 1 mM CP was incubated with 10 □M Mn-porphyrins in the presence of 2 mM ascorbic acid in PBS at pH 7.4 and 5.5 at 37 °C under aerobic conditions (0.26 mM O_2).
Results: The most effective cyt P450-like catalyst was the highly electron-deficient Fe(III) *meso*tetrakis(2,6-difluoro-3-sulfonatophenyl)porphyrin (4.6% yield). All porphyrins were more effective at pH 5.5 than at pH 7.4.
Conclusions: Fe-porphyrin can hydroxylate (activate) CP at similar efficiency as cyt P450 does (2.6% yield). The observed pH effect could be of importance in terms of specificity since tumor extracellular pH was reported to be as low as 5.2 and near the surface of macrophages to be as low as 3.6.
Antioxidant potency:
Methods: MnTnHex-2-PyP is a lipophilic (hexyl-) analogue of MnTE-2-PyP (ethyl-, highly potent SOD mimetic) which we tested for its efficacy in stroke model: 5 min after 90 min of middle cerebral artery occlusion, rats were given 75 or 225 □g/kg subcutaneous bolus of MnTnHex-2-PyP, followed by twice daily injections for 7 days. MnTnHex-2-PyP levels were determined in plasma and brain by LC/MS/MS.
Results: MnTnHex-2-PyP was found at 8:1 ratio in plasma:brain, while it was 100:1 for hydrophilic MnTE-2-PyP. Neurologic scores were dose dependently improved. High dose was without adverse effects. Rats in that group gain on average 14 g while they lost 17 g in saline group. Total infarct size was reduced significantly in high-dose group (30%, p=0.02).
Conclusion: Observed dose-dependent protective effect of MnTnHex-2-PyP in stroke is an important result as there are still no effective treatments for this ailment.
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Inter-kingdom cell-to-cell inhibitors: a novel target for antimicrobial drugs

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Background: The worldwide challenge of antimicrobial resistance and the paucity of novel antibiotics underscore the urgent need for innovative therapeutics. The increasing understanding of bacterial pathogenesis and inter-cellular communication, when combined with contemporary drug discovery tools and technologies, provides a powerful platform for translating basic science into therapeutic applications to combat bacterial infections. Inter-kingdom chemical signaling bridges the communication between bacteria and their hosts. Many bacterial pathogens rely on a conserved membrane sensor, QseC, to sense and respond to host adrenergic signaling molecules and to bacterial signals to promote expression of virulence factors.
Methods: We used a combination of high throughput screen of small molecules, qRT-PCR, and virulence tests to identify QseC inhibitors.
Results: Here we show that small molecule inhibitors of QseC-mediated signaling markedly inhibit the virulence of several pathogens in vitro and in vivo in animal models. We identified a potent small molecule, LED209, which inhibits binding of signals to QseC, preventing QseC's autophosphorylation, and consequently inhibiting QseC-mediated activation of virulence gene expression in enterohemorrhagic E.coli (EHEC), Salmonella typhimurium and Francisella tularensis. LED209 also prevented formation of lesions on epithelial cells by EHEC, and F.tularensis survival within macrophages. Remarkably, LED209 treatment protected mice from lethal S.typhimurium and F.tularensis infection. LED209 is not toxic and does not inhibit pathogen growth.
Conclusions: Inhibition of microbial virulence without inhibition of growth may engender less selective pressure to promote the generation of resistance. As demonstrated herein, inhibition of inter-kingdom inter-cellular signaling constitutes a novel and highly effective strategy for the development of a new generation of broad spectrum antimicrobial agents.

The Uncertain Future or the End of Modern Medicine?: A Clinicians View of Antibiotic Resistance-Way to Prevent the Inevitable

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As a clinitian I am rapidly approaching a time when informed consent to a young healthy adult about to have a minor operation I will have to say –"you may get infected with an organism for which there is no treatment and which may kill you"
We got to this state in three simple ways:
1-antibiotic overuse
2-antibiotic misuse
3-antibiotic abuse
This led to work that resulted in a paper for debate viz: M Spigelman 2005. MRSA Why treat the symptoms and not the disease? Annals of The Royal College of Surgeons of England 87: 6: 452 – 453). This was followed up by 3 articles detailing some of the research we are doing and the ideas we have developed to try to control this modern scourge.
The tackling of the increasing spiral of microbial resistance has been to ignore the real cause and try to limit infections-by various measures based on containment.
Our rearch shows certain areas where improvements can be made to contain cross infection paricularly in one case where we believe probiotics will be of use both as a cleaning disinfactant material and as a soap.
My presentation will highlight areas where we can make possible improvements to contain the development and spread of microbial resistance both in the community and in hospitals. How use in selected cases of pro-biotics can be beneficial in this as we have to learn how to handle bacteria from the source. As well the abuses of antibiotics in the community and food industry which has to be tackled with urgent priority.

Search for the Perfect Synthetic Isoprenoid-Glycolipid Vaccine Adjuvant

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Background: Subunit vaccines capable of providing protective immunity against the intracellular pathogens and cancers that kill millions of people annually require an adjuvant capable of directing a sufficiently potent cytotoxic T lymphocyte response to purified antigens, without toxicity issues. Archaeosome lipid vesicles, prepared from isoprenoid lipids extracted from archaea, are one such adjuvant in development. Here, the stability of an archaeal core lipid 2,3-di-O-phytanyl-sn-glycerol (archaeol) is used to advantage to synthesize a series of disaccharide-archaeols and show that subtle variations in the carbohydrate head group alters the type and potency of immune responses mounted in a mammal.
Methods: The archaeal core lipid 2,3-di-O-phytanyl-sn-glycerol (archaeol) was prepared from Halobacterium salinarum lipids, and used as a precurser to synthesize various glycoarchaeols. These lipids were combined with phospholipid to form antigen-containing archaeosomes to test for adjuvant activity in animal trials. Mice (6/group) were injected twice subcutaneously. Cytotoxic T cell activity in splenic cells was quantified 8 weeks from first injection.
Results: Critically, a glycosylarchaeol was required in the archaeosome formulation to elicit high cytotoxic $CD8^+$ T cell activity in mice, with highest responses to antigen entrapped in archaeosomes containing disaccharides of glucose in β or α 1-6 linkage (β-gentiobiose, β-isomaltose), or of β-lactose.
Conclusions: 1) This first study on synthetic archaeal lipid adjuvants reveals potential for this class of regulatory friendly, easily scalable, inexpensive and potent glyco-adjuvant. 2) The structural detail of the glycosyl head group altered adjuvant activity. 3) The synthesis strategy using biosynthetic archaeol and carbohydrate precursors was simple. 4)The structural possibilities of archaeal glycolipids for synthesis and adjuvant testing are numerous, but may ultimately lead to design of effective and synthetic adjuvants tailered to provide an optimal immune response to protect against specific pathogens.

Penetration of Bioactive Proteins and Peptides across Stratified Mucosae in a Porcine Ex-Vivo Model

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Background: The non-keratinized stratified mucosa lining the oral cavity, esophagus and vagina provide relatively permeable surfaces to deliver therapeutic agents to treat local and systemic diseases. On the other hand, if micro-organisms and their toxins present at these surfaces penetrate the tissues they can lead to pathological conditions ranging from periodontal disease to toxic shock syndrome and AIDS. This presentation describes the use of a porcine ex-vivo model to study mucosal permeability and tissue pathogenesis.
Methods: Pig mucosal tissue provides an excellent model for human for drug delivery and permeability studies (Squier C.A., et al., J Pharm. Sci. 97:9-21, 2008). Specimens of pig buccal and vaginal mucosa were removed at slaughter and placed in thermostatted continuous flow mucosal perfusion chambers for up to 12 hours (Squier C.A., et al. J Pharm Sci 86:82-84, 1997). Our studies examined the flux of a bioactive peptide, I^{125} labeled transforming growth factor β (TGFβ) across buccal mucosa in the presence of phosphate buffered saline (PBS) or the mucoadhesive polymer, chitosan. In separate experiments, the flux of S^{35} labeled staphylococcal toxic shock syndrome toxin (TSST-1) across vaginal mucosa was determined in keratinocyte serum-free medium (KFSM) medium alone or with the addition of 50µg/ml of a staphylococcal virulence factor, α-hemolysin. Experiments utilized at least 7 replicates for each of the conditions.
Results: Flux of TGFβ was significantly ($p<0.05$) increased in the presence of chitosan (241±96 dpm/cm^2/min) compared to PBS (36±18 dpm/cm^2/min) suggesting that the mucoadhesive may provide higher local concentration of compound at the mucosal surface than does a solution. The flux of TSST-1 across the vaginal mucosa in the presence of α-hemolysin (2.7±0.8) was significantly ($p<0.05$) greater than that in medium alone (1.3± 0.4 ng/cm^2/min) and was associated with marked histological changes in the epithelium, including massive lymphocytic infiltration and separation of epithelium from connective tissue, suggesting a synergistic role for virulence factors in the penetration of bacterial toxins.
Conclusions: the use of porcine tissue ex-vivo provides a realistic model for studying the kinetics and pathogenesis of tissue penetration by bioactive peptides and pathogenic toxins in real time for up to 12 hours.

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17-year Survey of Triclosan Efficacy on Supragingival Plaque

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Background: Dentifrices and other oral hygiene preparations maybe formulated with triclosan (TCN), a broad-spectrum antimicrobial agent. The broad spectrum activity of TCN on a wide range of oral organisms including pathogenic species of oral bacteria has been demonstrated in laboratory studies. In clinical studies, subjects using TCN dentifrice demonstrate improved oral health due to significant reductions in dental plaque and gingivitis. This study examined the antimicrobial efficacy of TCN on human supragingival plaque over 17 years.
Methods: Adults who had not received dental treatment or systemic antimicrobial therapy for the previous 30 days were included. Data obtained from 105 adults over 12 separate study periods are presented in this report. Supragingival plaque was collected from the entire dentition and aliquots of the bacterial suspension were distributed onto solid media containing 0, 7.5 or 25 µg/ml TCN. Following incubation at 37°C for 5-7 days, the number of colony forming units (CFU's) was enumerated.
Results: All subjects demonstrated large numbers of CFU's on media without triclosan. By contrast, supragingival plaque bacteria were substantially inhibited by TCN ($p<0.001$). Inhibition with 7.5 and 25 µg/ml TCN ranged from 97.3-100% and 99.6-100%, respectively. For each TCN concentration, ANOVA was used to compare the percent susceptible bacteria between each study period. Over the 17-year duration, no differences in microbial susceptibilities were observed between each period for either concentration of TCN ($p>0.28$). Regression analyses for each TCN concentration indicated no alterations or trends in efficacy (p values of 0.208 and 0.71 for the 7.5 and 25 µg/ml TCN, respectively) over time.
Conclusions: The data demonstrates the significant efficacy of triclosan on supragingival plaque bacteria over a 17-year period with no change in antimicrobial susceptibility. Since study participants were not selected based on the use of any oral hygiene formulation, the results indicate that triclosan is efficacious on supragingival dental plaque from a variety of subjects. These results demonstrate that triclosan represents a safe and effective agent for dental plaque bacteria.

Recombinant Gas Vesicles: A Novel Display/Delivery System for Peptide Antigens (SIV)

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Background: The goal of this research was to: 1) Extend demonstration of evoked immunologic memory in the absence of an exogenous adjuvant to diverse gene sequences by testing recombinant gas vesicles (r-GV) displaying peptides encoded by different SIV genes (tat, rev or nef). 2) Evaluate the resulting r-GV for specific antigenic and immunogenic capabilities.
Methods: The generation of transformed Vac^- (minus), halobacterial mutant strain SD109 lacking the GV gene cluster, used a series of recombinant plasmids. Aliquots of isolated wt-GV and r-GV were electrophoresed using SDS-PAGE gels and the SIV peptides expressed as part of the r-GvpC protein identified with monkey anti-SIV/SHIV sera using Western blots. Mouse anti-SIV sera raised against SIV-DNA insert sequences were generated by immunizing BALB/c mice. To test immunogenecity four month-old mice (3 mice per each gene segment) were injected initially using low dose immunization (1-3µg total protein), boosters then used 50 µg total proteins per mouse. Sera were collected at 2 and 4 weeks after primary immunization and 4 weeks following the first booster. After the 2nd booster, sera were collected at 2, 4, 8, 12 and 17 weeks. Mice were re-immunized and sera collected 10 days later. The animals retained for a total of 43 weeks post-re-immunization. All sera were subsequently adsorbed and assayed by ELISA.
Results: Each of the three different exogenous DNA sequences had been stably retained in the recombinant gvpC gene. The transformations with each of the selected recombinant plasmids were successful and had conferred a functional Vac^+ status. Antisera from mice immunized with r-GV and also monkey anti-SHIV sera each recognize the chimeric r-GvpC protein.
Conclusions: 1) Different recombinant gvpC-SIV genes will support the biosynthesis of chimeric GvpC fusion proteins and generate functional organelles. 2) Monkey antibody elicited by in vivo infection with SIV recognizes these expressed SIV sequences as SIV peptides. 3) Test of antiserum elicited by immunizing mice with r-GV demonstrated notable and long term antibody titers. Continued presence of elevated antigen specific antibody is a feature critical to immunization based protection. The observed level of humoral responses and the maintenance of elevated responses to encoded peptides are consistent with the suggestion that in vivo there may be natural and slow release of epitopes over time.

Plumbagin: A Candidate for Targeted Anticancer Therapy

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Background: There are emerging reports on plumbagin documenting evidence of antitumor function in experimental systems. It mainly operates through modulation of redox potential mediated cellular function, particularly affecting the DNA repair pathway. Earlier studies show that plumbagin known to generate ROS and has been found to inhibit the activity of topoisomerase II (Topo II) through the stabilization of the Topo II-DNA cleavable complex. On the basis on these data, we presumed that BRCA1 blocked cells may be more sensitive than the corresponding control cells against plumbagin. Our expereiments with BG-1 ovarian cancer cells indeed proved this.
Methods:.BG-1 ovarian cancer cells antisensly blocked for BRCA1 was the cell line used for this study.
Results: The dose of plumbagin needed to kill 50% was 5.1 □M in the control cells and 2.68 □M for the BRCA1 blocked cells indicating that the latter was about two fold more sensitive to plumbagin than the wild type cells. Plumbagin was also found to generate reactive oxygen species (ROS) in cultured cells. Plumbagin can bind to the active site of ER-□ inducing ER-□ 46 kDa truncated isoform, which was found abundantly preempted in the cytoplasm compared with a 66-kDa full-length isoform. The truncated isoform is known to inhibit classical ER-□ signaling pathways. SiRNA transfected cells for ER-□ exhibited lower cytotoxicity upon plumbagin treatment than the control-transfected cells. We have done molecular mechanism of action of plumbagin in BRCA1 blocked ovarian cancer cell line by suppression subtractive hybridization and microarray.
Conclusions: This throws light on the fact that plumbagin may have a chemotherapeutic potential as an anticancer agent in BRCA1 defective breast/ ovarian cancer patients.

Acetylsalicylic Acid: an Immune-Modulator in HIV Infection

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Background: Management of AIDS in a country when more than 80% of population lives in deep poverty is a challenge, which no clinician would like to experience. To begin with lack of water and sanitation, not to mention nutritional depravation makes Anti-retrovirals an impossible choice. Acetylsalicylic Acid, being an inhibitor of Nuclear Factor-kappa beta (NF-$\kappa\beta$), may prove to be a cheap and relatively safe immune-modulator.
Methods: We randomized 20 drug naïve HIV-positive men and women into placebo and 1,2g of Acetylsalicylic Acid. All participants were supported by nutritional program with three meals per day. Baseline CD4 count, viral load (VL), and plasma levels of HIV-p24 antigen, Tumour Necrosis factor-alpha (TNF-α), Interleukin -2 (IL-2), IL-4 and IL-6 were measured 3-monthly for 12 months.
Results: We observe a decrease in the median CD4 count in the placebo group from 267 to 221 cells/mL and an increase in CD4 count in Acetylsalicylic Acid group from 364 to 437 cells/mL (p=0.015). The mean VL increased in placebo by 0.46 log compare with Acetylsalicylic Acid by 0.15 log. Participants with detectable p24 antigen increase from 19 to 71% in placebo. The p24 antigen remained negative in all who started off negative in Acetylsalicylic Acid arm. Mean plasma TNF-α levels were unchanged in the placebo but decreased from 11.31 pg/mL to undetectable in all in Acetylsalicylic Acid group. IL-6 levels decreased in the placebo arm but increased in the Acetylsalicylic Acid arm. IL-2 levels decreased in all but one in placebo; there was a decline from 7.64 to 5.67 pg/mL in the Acetylsalicylic Acid group. IL-4 increased from 59 to 186 pg/mL in the treatment arm.
Conclusions: This pilot study shows a beneficial increase in CD4 cell count following ingestion of Acetylsalicylic Acid. There is a lesser impact on the viral load. The cytokine studies that show a decrease in TNF-α and IL-2 and increases in both IL-4 and IL-6 suggest that the effects are in part cytokine mediated. We believed there is an urgent need to investigate these findings in a larger population.

Rabies in Bats and Protective Treatments of Humans in Europe

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Background: Surveillance of rabies in bats across Europe during the last 50 years revealed rabies infection in bats in many European countries and also some human cases after exposure to rabid bats. Humans should be treated against rabies pre-exposure and post-exposure. There is a need to protect some professions and travellers as well. According to WHO, rabies control needs to be adapted on the rabies surveillance in each country and rabies pre-exposure and post-exposure treatment of humans needs to assume bat rabies as well. Goals: 1) Find out if post-exposure vaccination against rabies is implemented in particular countries; 2) Find out if preventive treatments of bat researchers and cavers are used as well.
Methods: Questionnaire was dispatched by mail to contributors of the Rabies Bulletin Europe from European countries. We asked questions concerning recommendation for post-exposure treatment after a bat bite and for pre-exposure treatment of bat researchers and cavers.
Results: 15 countries across Europe participated in the study: Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Iceland, Netherlands, Norway, Poland, Serbia, Slovenia, Switzerland and United Kingdom. Post-exposure treatment is more frequently practiced (13 of 15 countries) than pre-exposure treatment of bat researchers (11 of 15 countries involved in the study). Pre-exposure treatment of cavers is practiced only in Czech Republic, France and Poland.
Conclusions: 1) Distribution of European lyssa viruses in Europe is not well understood because only few countries have suitable monitoring of these infections; 2) Growing knowledge concerning bat rabies and availability of successful treatment with rabies vaccine and immunoglobulin is interesting to medical doctors, veterinarians, cavers and bat researchers; 3) Reasons for actualizing recommendation concerning treatment against bat rabies are the changing world with large tourist movement and investigation of epidemiological importance of emerging infectious diseases as well.

New Medicines, New Problems: Understanding the Failure of the Phase I Clinical Trial of Tgn1412

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In 2006 a near-fatal "cytokine storm" occurred in six healthy volunteers during the phase-I clinical trial of TGN1412, a therapeutic superagonistic CD28-specific monoclonal antibody, signalling a failure of pre-clinical safety testing. Subsequently we established that TGN1412 could stimulate a "cytokine storm" in vitro but only if correctly presented to human cells by immobilisation onto plastic or when added in the presence of endothelial cells. These novel procedures would have predicted the toxicity of this superagonist and are now being applied to emerging immunotherapeutics and to other therapeutics that have the potential to act upon the immune system. Data from these novel in vitro procedures suggests that the dose of TGN1412 given to human volunteers was close to the maximum immunostimulatory dose. In contrast to humans we found that TGN1412 is not superagonist for cells from the non-human primate model used for safety testing. A "cytokine storm" was not seen during pre-clinical in vivo safety testing as non-superagonistic CD28-specific monoclonal antibody is not pro-inflammatory for human or non-human primate cells.

A Common Molecular Basis For Ryanodine Receptor Dysfunction Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) And Malignant Hyperthermia (MH) Provides Opportunities For Novel Therapeutic Strategies

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Background: Missense mutations in RyR2 underlie the condition CPVT, which is associated with cardiac arrhythmias during exercise or stress. CPVT mutations occur in 3 clusters, corresponding to the N-terminal, central and the pore-forming regions of RyR2 (or regions 1, 2 & 3 respectively). A similar clustering of RyR1 mutations occurs in the skeletal muscle disease MH. Recent evidence suggests that (i) mutations in regions 1 and 2 occur within interacting domains and (ii) region 3 is actually another pair of interacting domains. If this hypothesis is correct, it should be possible to mimic the effects of specific mutations using RyR peptides, which act competitively to disrupt the interdomain interactions. Here we report the effects of a peptide (DPc10), which binds competitively to region 2 of RyR2, thereby disrupting interactions between regions 1 and 2.
Methods: Rats (Wistar, 200g) were humanely killed in accordance with UK legislation and ventricular myocytes isolated enzymatically. Cells were permeabilized with saponin (5 µg/ml) and perfused with a mock intracellular solution containing (mM) ATP 5, phosphocreatine 10, HEPES 15 (free Ca^{2+} 200 nM, Mg^{2+} 1 mM. pH 7.1, 21°C) Solutions also contained fluo-3 (10 µM), allowing changes in cytosolic [Ca^{2+}] to be detected using confocal microscopy.
Results: In permeabilized ventricular myocytes, 50 µM DPc10 induced (i) a transient increase in resting Ca^{2+} spark frequency (ii) a sustained increase in the RyR mediated Ca^{2+} leak, which could be revealed by inhibition of the SR Ca^{2+} pump and (iii) a decrease in the cytosolic [Ca^{2+}] threshold for spontaneous Ca^{2+} waves. However, a similar peptide (DPc15-mut) containing a disease mutation (A2474S) linked to CPVT had no effect on RyR2 function.
Conclusions: 1) The decreased threshold for spontaneous SR Ca^{2+} release induced by DPc10 is likely to be proarrhythmic and therefore consistent with the disease phenotype. 2) The absence of effect of DPc10-mut can be explained if the disease mutation impairs the ability of the peptide to interact competitively with region 1. 3) The ability to mimic the effects of disease mutations in normal cells using targeted peptides provides an opportunity for the development of novel therapeutic strategies.

Data review on Medicines utilization and expenditure in Serbia from 2004-2007

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Aim: The aim of this paper is to provide the review of the total consumption as well as financial means spent for medicines in Serbia from 2004-2007. Generally over the past decades, medicines have made a major contribution to improving the health status of patients. At the same time, pharmaceuticals expenditure has increased rapidly. Spending on medicines is outpacing economic growth. In respect of Serbia, it has a population of around 7.5 million and a tradition of a public health care system financed through a social health insurance. The Republic Institute for Health Insurance (RIHI) is financed by social insurance contributions raised on individual salaries (12, 3% of gross salary) and shared equally by employers and employees. Prescription drugs account for about 11% of the RIHI spending on health care.
Method: ALIMS is authorised (by the Law on Medicines and Medical Devices "The Official Gazette of the Republic of Serbia", 84/2004 and 85/2005-the other law) for collecting and processing data on medicines marketing and consumption. Data on medicines marketing from 2004-2007 were gathered from obliged entities and processed by the DDD/1000 inhibitant/day methodology and ATC classification. Financial analysis was done as well.
Results: It was established that the total medicines marketing in Serbia in 2004 was €340 million, in 2005 it was €380 million, in 2006 it was €510 million and in 2007 it was €690 million. Since the total DDD has been around 1000, the consumption data have shown that one drug has been used by each inhabitant every year in Serbia. In all four years, medicines for cardiovascular disorders (group C) have been at the first place. By processing consumption data for group C as DDD, from 2004-2007 consumption was 331, 334, 388 and 400 DDD/1000 inh./day, respectively.
Conclusion: In era of ageing population and rising health care costs, analyses and statistics on medicines market make an important indicator in health care. Combined with epidemiological data, it can provide a scientific contribution to the efficient, efficacious and safe use of medicines. Health care professionals and policy makers seek for implementation of effective pharmaceutical policies that would support further health improvements by introducing new and more effective medicines, whilst containing pharmaceuticals expenditure.

Dipeptidyl peptidase 4 inhibitors: a new class of oral agents for the treatment of type 2 diabetes mellitus

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Type 2 diabetes mellitus is a major health problem in the 21st century. New therapies are needed to control metabolic abnormalities, and also to preserve β-cell mass and to prevent loss of β-cell function. Glucagon-like peptide 1 (GLP-1) is a drug candidate which potentially fulfils these conditions. Mice lacking dipeptidyl peptidase IV are protected against obesity and insulin resistance.
Several DPP-4 inhibitors are in clinical development; these are orally active and increase levels of active GLP-1, which in turn increases insulin secretion and reduces glucagon secretion and thereby lowers glucose levels. Most experience exists for sitagliptin and vildagliptin, which both have a long duration of action, allowing once-daily administration.
DPP-4 inhibition is an efficient treatment of type 2 diabetes, both as monotherapy and combination therapy. Because of its efficiency, safety, and tolerability in association with the oral mode of administration, it is expected that DPP-4 inhibition will be a first-line treatment of the early stage of type 2 diabetes, particularly in combination with metformin or thiazolidinediones.
The DPP-4 inhibitor vildagliptin improves insulin sensitivity and β-cell function, leading to improved postprandial glycemia in subjects with IFG, who are known to have β-cell dysfunction. Thus, vildagliptin may prevent progression to diabetes in high-risk subjects.
Incretin mimetics are a new class of pharmacological agents with multiple antihyperglycemic actions that mimic several of the actions of incretin hormones. Exenatide is the first incretin mimetic approved for clinical use. Exenatide therapy can be considered as an alternative to insulin in patients with treatment failure on metformin monotherapy or on metformin together with a sulfonylurea.
Conclusion: The long-term consequences of DPP-4 inhibition on ß-cell function and the durability of glucose lowering achieved with sustained DPP-4 inhibition require careful clinical assessment. It seems prudent to pursue additional detailed studies of the biological role(s) of DPP-4 and the consequences and safety of highly selective DPP-4 inhibition in experimental and clinical models of diabetes.
Therapy for diabetes will probably not alter radically in the next few years unless long-term data demonstrate other advantages over metformin and insulin. However, agents modulating GLP-1 are likely to be playing a major role in combating the world-wide burden of diabetes in the 21st century.

Which ABC-transporters should we target in leukemia?

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ABC-transporters are a large family of proteins involved in active transport across biological membranes. Some members of this family cause drug resistance in malignant diseases via ATP dependent drug efflux from malignant cells. This phenomenon was intensively analyzed in leukemia.
ABCB1 (P-g) and ABCG2 (BCRP) were shown to be associated with poor response to chemotherapy in adult acute myeloid leukemia (AML). Both proteins confer resistance to a wide range of chemotherapeutic drugs in vitro. Therefore, they represent possible therapeutic targets. In pediatric AML, this is the case for ABCG2 but not for ABCB1. In acute lymphoblastic leukemia (ALL), both proteins appear less relevant with the probable exception of ABCB1 in adult patients.
ABCC3 (MRP3) has a strong prognostic impact in AML and ALL independent of age group. However, ABCC3 does not cause much drug resistance in vitro. Therefore, it remains to be elucidated whether its correlation with poor response to therapy is causative or just an epiphenomenon.
ABCA3 might be an additional cause of drug resistance in AML. It is associated with in vitro drug resistance and response to therapy. Interestingly it causes drug resistance via intracellular drug sequestration instead of drug efflux from the malignant cell.
Specific inhibitors of ABC-transporters can sensitize leukemic cells to chemotherapy. For some types of leukemia it would be desirable to develop drugs that inhibit a set of ABC-transporters.

The Pathogenesis of Autoimmune Diseases: New Possibilities for Drug Development

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With their overall prevalence of 5-8 %, the approximately 80 defined autoimmune diseases comprise a major burden to public health. They have many common features and are increasingly recognized as a group of related illnesses that should be studied collectively as well as individually. Unfortunately, efforts to prevent or treat these conditions are hampered by a limited knowledge of the pathogenesis. In 1997, a German group demonstrated that the antigen of the biomarker EMA (endomysial antibodies) in coeliac disease (CD) is a calcium-dependent thiol enzyme, transglutaminase 2 (TG2). This important discovery opened up an exciting field of research aimed at a better understanding of the pathogenesis of CD. Progress has already improved the opportunities for laboratory diagnostics, and hopefully, new ways of treating and preventing CD will become available. This presentation will highlight some of the intriguing mechanisms of the pathogenesis of CD, such as the structure of the neo-antigen, the involvement of calcium and zinc, and the effects of CD-antibodies on TG2 activity.
In another common inflammatory autoimmune disease, rheumatoid arthritis (RA), antibodies against peptide-bound citrulline constitute a specific biomarker. The enzymes catalyzing citrullination, the peptidylarginine deiminases (PADs), share many features with the TGs. Moreover, auto-antibodies against PAD can be detected in RA. Thus, in two major T-cell restricted autoimmune diseases, antibodies can be detected against a posttranslationally modified protein as well as against the calcium-dependent thiol enzyme responsible for the substrate modification. Reflecting the progress in the field of CD, this presentation will also focus on the fascinating mechanisms which may be involved in the pathogenesis of RA. Moreover, the many pitfalls due to dubious laboratory practice will be addressed together with the great potential when a fundamental biological mechanism is understood at the molecular level.

Staphylococcal Enterotoxin Vaccines: Future Utility for Fighting Staphylococcus aureus?

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Background: There is little doubt that Staphylococcus aureus is as much today a microbial menace as it was when first discovered by Alexander Ogston in the late 19th century. With rising rates of antibiotic resistance evident now in the 21st century, it seems clear that additional measures must be made to counter S. aureus. Past efforts of pacifying this pathogen and its myriad virulence factors have included the generation of recombinantly-attenuated versions of staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin 1 (TSST-1). Naturally, these toxins are superantigens that bind to major histocompatibility complex II and specific T-cell receptors. These protein toxins stimulate T cells that then release unhealthy levels of pro-inflammatory cytokines throughout the host. Ultimately, such events can cause toxic shock. Vaccine constructs, in particular those towards SEB and TSST-1 used in our studies, were devoid of receptor-binding capabilities and thus rendered non-toxic. The major goal of our studies was to show protection against a toxin challenge in various animal models. Hopefully, these studies will translate into human protection against S. aureus-induced toxic shock and thus provide clinicians with additional tools against disease.
Methods: A mouse model was first developed to screen potential vaccine candidates. This model exploits the natural synergy that exists between superantigens and lipopolysaccharide derived from Gram-negative bacteria. After extensive mouse testing, a non-human primate model was also used for establishing vaccine efficacy. Vaccine constructs were evaluated by various routes of administration which include intraperitoneal, intramuscular, and intranasal. Animals were challenged with a lethal dose of toxin administered into either the peritoneal cavity or lungs.
Results: Various animal studies, employing mice and non-human primates, revealed excellent efficacy of these protein vaccines against a lethal toxin challenge.
Conclusions: Recombinantly-attenuated versions of S. aureus superantigens represent worthy, non-toxic targets for vaccination. Multiple studies from various groups suggest promising efficacy of superantigen-based vaccines towards specific toxins and the microbe; however, further studies await results from human clinical trials.

Transdermal Delivery of Naltrexone, In Vitro Testing to Human Volunteers

STINCHCOMB AL

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Background: Alcoholism and opiate addiction lead to major health-related problems and societal costs for people around the world. Therefore, research and development for improved pharmacologic treatments for drug and alcohol abuse are very important. Naltrexone, an opioid antagonist, is currently used in oral tablet form to help maintain opioid addicts in a drug-free state. Most recently, naltrexone has been indicated as an adjunct in the treatment of alcohol dependence, as well as reported to reduce alcohol craving in certain alcoholic populations. Transdermal delivery of naltrexone is desirable for opioid addicts and alcoholics in order to help reduce side effects associated with oral therapy and improve compliance. Naltrexone itself does not have the essential physicochemical properties that would allow a therapeutic dose of the drug to cross the human skin barrier.
Methods: For the last 12 years we have been designing and synthesizing prodrugs, which are more skin permeable than naltrexone, in order to make a therapeutically successful drug delivery system. We have hypothesized that prodrugs of naltrexone and prodrugs in combination with microneedle treatment will improve the transdermal delivery rate of naltrexone. These prodrugs have made excellent research tools for investigating quantitative structure-permeability relationships (QSPR) for transdermal flux and optimization of flux in combination with microneedle enhancement.
Results: These prodrugs/microneedles have improved naltrexone delivery rates across the skin because of optimized physicochemical properties for faster diffusion. Testing has been completed to measure the drugs' penetration and concurrent bioconversion through human skin in vitro with and without microneedle use, and to examine the pharmacokinetics of the drugs in guinea pigs in vivo with and without microneedle use. Correlation of our in vitro data with the in vivo model allowed us to design and complete a human proof-of-concept study using microneedles and naltrexone hydrochloride.
Conclusions: We have been able to improve the flux of naltrexone across the skin up to 10-fold with these methods. The current focus of our studies is to increase the rate of naltrexone transdermal delivery by combining microneedle technology with a prodrug and formulation approach. Funding:NIHR01DA13425.

Extensive Necrosis of Visceral Melanoma Metastases after Immunotherapy

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Background: The prognosis for metastatic melanoma remains poor even with traditional decarbazine or interferon therapy. 5-year survival is markedly higher amongst patients undergoing metastatectomy. Unfortunately not all are suitable for metastatectomy. Alternative agents for systemic therapy have, to date, offered no greater rates of survival beyond traditional therapy. A toll-like receptor 9 agonist, PF-3512676 (formerly known as CPG 7909) is currently being evaluated for its potential.
Case presentation: We present the case of a 54-year-old Caucasian male with completely resected metastatic cutaneous melanoma after immunotherapy. The patient initially progressed during adjuvant high-dose interferon, with metastases to the liver, spleen, and pelvic lymph nodes. During an 18-month treatment period with PF-3512676 (formerly known as CPG 7909), a synthetic cytosine-phosphorothioate-guanine rich oligodeoxynucleotide, slow radiologic disease progression was demonstrated at the original disease sites. Subsequent excision of splenic and pelvic nodal metastases was performed, followed by resection of the liver metastases. Histologic examination of both hepatic and splenic melanoma metastases showed extensive necrosis. Subsequent disease-free status was demonstrated by serial positron emission tomography (PET).
Conclusions: Existing evidence from phase I/II trials suggests systemic treatment with PF-3512676 is capable of provoking a strong tumor-specific immune response and may account for the prolonged tumor control in this instance.

The Inverse Relationship between Cisplatin and Paclitaxel Resistance: Two 'Magic Bullets' are Needed

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Background: Cisplatin and paclitaxel have both been successfully used for the treatment of solid tumours, however the majority of patients will relapse with resistant disease. Work in our laboratory and preliminary reading of the literature suggested that cisplatin-resistant cell lines were often sensitive to paclitaxel and the reverse was also true, paclitaxel-resistant cells were often sensitive to cisplatin. The objective of our systematic review was therefore to 1) To examine the pre-clinical evidence for an inverse resistance relationship between cisplatin and paclitaxel and 2) The clinical evidence for the use of paclitaxel salvage chemotherapy in patients with cisplatin-resistant cancer.
Methods: Medline was searched for 1) Cell models of acquired resistance reporting cisplatin and paclitaxel sensitivities and 2) Clinical trials of single agent paclitaxel salvage therapy for cisplatin/carboplatin-resistant ovarian cancer.
Results: Our systematic literature review found 137 models of acquired drug resistance. 68.1% of cisplatin-resistant cells were sensitive to paclitaxel and 66.7% of paclitaxel-resistant cells were sensitive to cisplatin. A similar inverse pattern was observed for cisplatin vs. docetaxel, carboplatin vs. paclitaxel and carboplatin vs. docetaxel. These associations were independent of cancer type, agents used to develop resistance and reported mechanisms of resistance. Sixty-five eligible clinical trials of paclitaxel-based salvage after platinum therapy were identified. Studies of single agent paclitaxel in platinum-resistant ovarian cancer where patients had previously received paclitaxel had a pooled response rate of 35.3%, n = 232, compared to 22% in paclitaxel naive patients n = 1918 ($p < 0.01$, Chi-squared). Suggesting that pre-treatment with paclitaxel may improve the response of salvage paclitaxel therapy. The response rate to paclitaxel/platinum combination regimens in platinum-sensitive ovarian cancer was 79.5%, n = 88 compared to 49.4%, n = 85 for paclitaxel combined with other agents ($p < 0.001$, Chi-squared), suggesting a positive interaction between taxanes and platinum. Therefore, the inverse relationship between platinum and taxane resistance seen in cell models is mirrored in the clinical response to these agents in ovarian cancer. An understanding of the cellular and molecular mechanisms responsible would be valuable in predicting response to salvage chemotherapy and may identify new therapeutic targets.
Conclusions: While neither cisplatin or paclitaxel may be a universal 'magic bullet' on their own, the correct sequencing between these two agents may lead to improved response rates particularly in ovarian cancer.

Daptomycin: Old drug, New data, Endless Puzzles

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Background: Daptomycin is a potent antibiotic that displays rapid bactericidal activity and has recently been approved by the FDA to treat complicated skin infections. It is a cyclic anionic tridecapeptide, with a number of D-amino acids (D-asparagine, D-alanine, and D-serine), 3 uncommon amino acid residues (ornithine, (2S,3R)-3-methyl-glutamic acid and kynurenine), and a N-terminus that is acylated with a n-decanoyl fatty acid side chain. It functions in a calcium-dependent manner. Aim: To understand its mode of action, specifically its interaction with 1) calcium and 2) bacterial model membranes.
Methods: To understand the effect of calcium on the structure of daptomycin, we have performed analytical ultracentrifugation experiments, as well as characterized the structure of daptomycin in the presence of Ca^{2+} and Mg^{2+} by nuclear magnetic resonance (NMR). To determine to what extent daptomycin perturbs membranes, we have performed differential scanning calorimetry, fluorescence, and NMR experiments.
Results: Our NMR and other biophysical data suggest that daptomycin forms a micellar structure in solution when Ca^{2+} is present in a 1:1 Ca^{2+}/daptomycin ratio and that this binding event, as well as binding to lipid membranes is not accompanied by as significant a structural change as originally postulated in Jung et al., Chem Biol. 2004 Jul;11(7):949-57. Our data also suggest that the presence of negatively charge phosphatidylglycerol and Ca^{2+} are essential for membrane interaction and perturbation (by fusion).
Conclusions: The implication of these results for the mechanism of action of daptomycin will be discussed.

Ocular Infections: Impact of Resistance to Fluoroquinolones and Other Antibiotics on Topical Therapies

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Background: Ocular infections of the anterior segment include bacterial conjunctivitis and keratitis. Both of these infections are treated with topical antibiotic drops. Ophthalmic antibiotic drops include aminoglycosides, tetracyclines, chloramphenicol, fusidic acid, combinations of two or more antibiotics and most recently in the last 15 years, fluoroquinolones (FQs). An important question is whether or not acquired resistance to an antibiotic limits the efficacy of the corresponding ophthalmic products.
Methods: The clinical and microbiological efficacy was correlated with in vitro susceptibility of the pre-therapy pathogens in Alcon-sponsored clinical studies with FQs conducted during the past decade.
Results: Clinical and microbiological efficacy results from more than 3000 conjunctivitis patients revealed that frequency of treatment failures due to a specific pathogen were directly proportional to the frequency of occurrence of the specific pre-therapy bacterial species. Generally, the pre-therapy pathogens from treatment failure patients were susceptible to FQs and did not change their FQ susceptibility as a result of FQ therapy.
Conclusions: Antibiotics applied topically that are rapidly bactericidal lead to more rapid resolution of clinical signs and symptoms and eradication of pathogens than antibiotics that are bacteriostatic. FQ ophthalmic products are formulated with very high concentrations (3000 – 5000 mcg/ml) relative to the FQ MICs for pathogens, even those that have become resistant to FQs. Treatment failures due to FQ resistant pathogens did not occur more frequently than treatment failures due to FQ susceptible pathogens indicating equivalent efficacy for FQ susceptible and resistant pathogens. Moreover, in no case did the FQ susceptibility of a pre-therapy pathogen change as a result of FQ therapy.

Fluoride and Aluminum Interactions: AlFx as the Magic Bullets Producing Aberration of G Proteins

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Background: The wide use of fluoride (F) and aluminum (Al^{3+}) in medicine, industry, and agriculture, started the era of supplementation of living environment with these ions as never before. Many effects primarily attributed to F are caused by its interactions with Al^{3+}. The effects of aluminofluoride complexes (AlFx) - new analogues of phosphate groups - have been demonstrated by many studies with proteins, intact cells, and whole animals. The widespread use of AlFx as a general activator of G proteins provided evidence that AlFx represent Magic Bullets for human body. The warning of the potential danger from the interactions of Al^{3+} and F to human health has emerged from research on cell signaling.
Methods: Biochemical and immunohistochemical methods were used for *in vitro* experiments. Meta-analysis techniques for human studies were used because only summary statistics are typically available in the literature.
Results: Fluoride affects the activity of numerous enzymes and various biological processes. The effective dose is much lower in the presence of trace amounts of Al^{3+}. AlFx interact with all known G protein-activated effector enzymes. Numerous findings demonstrate the positive correlation with the impairment of homeostasis, growth, development, cognition, and behavior. The predictability from *in vitro* data as well as the correlation with numerous epidemiological, ecological, and clinical studies from fluoridated communities will be presented. AlFx may induce most of the pathological hallmarks of autism and Alzheimer's disease.
Conclusion: 1) The definition of a safe concentration of F for humans must consider that the dose is much lower in the presence of Al^{3+}. 2) It is evident that AlFx is a molecule giving the false information, which is amplified by processes of signal transmission. Pharmacologists estimate that up to 60% of all medicines used today exert their effects through G protein signaling pathways. AlFx are man-made Magic Bullets that produce aberration of G proteins. 3) Assessment of the health risks linked to the F and Al^{3+} interactions would contribute significantly to the decline of many several disorders in the 21st century.

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Strunecka et al. Fluoride interactions. Curr. Signal Transd. Therapy 3, 190, 2007.

Strategic Role of Prednisolone, Vincristine and Asparaginase in Pediatric Leukemia as a Paradigm of Success in Oncology: Individualized Tumor Response Testing and Microarray Analysis

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Background: A significant progress has been obtained in pediatric acute lymphoblastic leukemia (ALL). In analysis of last 50 years, the cure rate increased from 1% to over 70%. Recent results showed even up to 90% of cure in subgroups of patients. Aims: The value and role of individualized tumor response testing (ITRT) to prednisolone, vincristine and asparaginase in pediatric ALL.
Methods: The in vitro drug resistance profile in 359 children with newly diagnosed ALL was determined by the MTT assay. All patients were stratified into 2 risk groups on the basis of white blood cell (WBC) count: patients with WBC>50G/L (n=77) were treated as high-risk group according to New York protocol, while all others (n=282) with BFM protocol for standard risk group. Patients with early deaths, bcr-abl rearrangement, in vivo prednisolone poor-responder, and infants were excluded from this study. Combined in vitro drug resistance profile to prednisolone, vincristine and asparaginase (PVA score) was determined for each patient.
Results: (1) Based on result of ITRT, all patients were divided into 3 groups: those, whose samples underwent spontaneous apoptosis were regarded as sensitive (S), those with PVA score 3-7 were regarded as intermediately sensitive (M) and those with PVA score 8-9 as resistant (R). With median follow-up of 20 months, probability of disease free survival (pDFS) was 0.90±0.03, 0.82±0.05 and 0.64±0.11 (p=0.023) for S, M and R groups, respectively. Within subgroups characterized by initial WBC count similar distribution was observed. (2) In multivariate analysis, two factors were significant: bone marrow response by day 33 (p<0.001) and PVA score (p=0.002) for the group of all patients. When standard-risk patients were analyzed separately, also PVA score (p=0.038) and bone marrow response by day 33 (p=0.031) were the only significant factors for pDFS. (3) Results of ITRT study were recently confirmed (by other groups) using microarray technology.
Conclusions: (1) Combined in vitro drug resistance profile to prednisolone, vincristine and asparaginase is a potent prognostic factor in childhood de novo acute lymphoblastic leukemia. (2) ITRT results were confirmed by microarray analysis.
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Induction of Complete Tumor Remission by DNA-Directed Alkylating Agents

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Background: DNA alkylating agents possess a number of drawbacks including high chemical reactivity and lack of intrinsic DNA binding affinity. To overcome these drawbacks, it is important to design and synthesize chemical stable alkylating agents with potent antitumor efficacy. Aims: 1) To synthesize DNA-directed alkylating agents by linking the phenyl N-mustard pharmacophore to DNA-affinic molecules (i. e., 9-anilinoacridines or quinolines) via a urea or carbamate linker. 2) To study structure-activity relationships. 3) To study the mechanism of action.
Methods: A series of DNA-directed alkylating agents, N-mustard-9-anilinoacridine and N-mustard-quinoline conjugates bearing a urea or carbamate linker were synthesized by reacting 4-[*N*,*N*-bis(2-chloroethyl)amino]phenyl isocyanate or 4-[bis(2-chloroethyl)amino] phenyl 4-nitro-phenyl ester with appropriate 9-anilinoacridines or 4-aminoquinolines in dry DMF under basic conditions.
Results: The newly synthesized derivatives exhibited significant cytotoxicity against human acute lymphoblastic leukemia and various solid tumors with IC_{50} in submicro molar range and have little or no cross-resistance to either taxol or vinblastine. Several derivatives are able to induce complete tumor remission in nude mice bearing human breast carcinoma MX-1 xenograft and maintain no relapse for a long period of time. Among these conjugates, BO-1051 exhibited potent cytotoxity against various human glioma stem-like cells growth in vitro and induced autophagy in these cells. Moreover, this agent significantly inhibits human glioma U87 xenograft. The new N-mustard derivatives are able to induce DNA interstand cross-linking in tumor cell and have long half-life in rat plasma.

Inhibitory Effects of Demecolcine on NK Cell Functions: Implications for Cancer Therapy

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Background: The cytostatic property of the microtubule-disrupting agent demecolcine has previously been exploited to some degree in oncology. Neoplasms of NK cells are generally highly aggressive. However, current therapies (chemotherapy and/or radiotherapy e.g.) are suboptimal and overall survival is dismal. Effects of demecolcine on NK functions were studied using the aggressive NK leukemia cell line KHYG-1 as a model.
Methods: KHYG-1 was treated with different demecolcine doses, 0.1 to 30 µM, for 12-15 hrs at 37 ^{o}C/ 5% CO_2, washed 3-4X in PBS and compared to untreated cells in subsequent experiments. Microtubules (anti α-tubulin) and cytotoxic granules (anti perforin) were imaged by confocal microscopy. Viability, Annexin V and 7AAD staining, and receptor expression levels were analyzed by flow cytometry and cytotoxicity measured in a flow cytometric assay. Degranulation (anti perforin) after target contact and phosphorylation of the kinase ERK were assessed by immunoblotting.
Results: In KHYG-1 cytolytic granules are constitutively polarized, normally a consequence of tumor target contact. Demecolcine treatment (10 µM) dispersed granules reversibly in about 90% cells. Cytotoxicity was significantly reduced at doses mentioned above, although, not completely abrogated. This was likely due to re-polarization of granules during assay duration as assessed in parallel imaging experiments. About 20-40% KHYG-1 were positive for Annexin V and about 10-20% positive for 7AAD at 0.1 to 30 µM doses. Degranulation after target contact was inhibited, expression of important activation receptors, NKG2D and NKp44, down-modulated to about 60%, and ERK phosphorylation to about 45%. In all experiments, a plateau effect at doses ≥1 µM was noted (Suck, G. et al. Int Immunol 2006, 18:1347-54).
Conclusions: Demecolcine significantly inhibited KHYG-1 functions and could be envisaged as a treatment for NK neoplasms, with the potential to induce apoptosis, interfere with cytotoxicity and proliferation as a cytostatic drug. A potential drawback is the reversibility of its effects. Combination therapy with the proteasome inhibitor Bortezomib, which has recently been shown to induce apoptosis in malignant NK cell lines, followed by hematopoietic stem cell transplant, is envisaged to improve this approach.

Recombinant therapeutic proteins - - - from the viewpoint of a biotechnologist

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Recombinant proteins are possible magic bullets for pharmacological interventions of the various diseases. However, it is challenging to find a protein with a desirable effect. Moreover, it is also tough to produce a recombinant protein of interest in a form ahieving full activity. We have explored pharmacologically relevant proteins using recombinant technologies.
Protein C is an anti-coagulant protein requiring gamma-carboxylation of the Gla domain for its activity. To ensure its sufficient modification we needed to produce recombinant protein C in mammalian cells. The culture conditions must be optimized to accomplish the satisfactory level of its productivity with enough gamma-carboxylation. Now protein C is on the market as an anti-septic agent from Eli Lilly.
Periostin was first identified as an ostoeblast specific factor-2 from cultured osteoblastic cells with expectation that it should exhibit the activity to stimulate osteoblast cell growth. In order to seek its pharmacological activity toward bone we produced periostin in a high yield by the use of a baculovirus expression vector system. Recently periostin gains much attention due to its potential to treat patients with heart diseases despite the expectation at its discovery.
Thus, the multidisciplinary expertise is required for the development of recombinant magic bullets.

Plasmodium Heme Crystallization: Methylene Blue to the Quinolines

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Background: Attempting to make quinine, WH Perkins created the aniline dye mauve, which provided the rational basis of staining microorganisms for inhibition. Ehrlich started from the aniline dye, methylene blue, to discover the first synthetic antimalarial proven effective in humans. Ehrlich later launched the first drug screen to identify compound 606 for syphilis. Methylene blue and the quinolines like chloroquine both bind heme and the heme crystal, hemozoin, to effectively kill Plasmodium parasites. Aims: 1) to show the mechanism of intracellular heme crystal formation and inhibition by methylene blue and the quinolines and 2) to compare inhibition of both P. falciparum and heme crystal inhibition by more than 2,000 existing drugs.
Methods: This study utilized subcellular fractionation, electron microscopy, mass spectrometry and heme crystallization assays to demonstrate heme crystal formation in Plasmodium. Different heme crystallizaton inhibition assays were compared to P. falciparum culture inhibition by the Johns Hopkins Clinical Compound Library, comprised of over 2000 approved drug molecules.
Results: P. falciparuim intracellular heme crystals were observed to grow within neutral lipid naonpheres by electron microscopy. Subcellular fractionation followed by mass spectrometry identified principally neutral lipids closely associated to heme crystals. The neutral lipids promoted efficient heme crystallization, inhibitable by the quinolines and methylene blue. The clinical compound drug screen inhibition of parasite and heme crystallization identified novel antimalarial activity of the antihistamine, astemizole, and the epidermal growth factor inhibitor, gefitinib.
Conclusions: Dye or quinoline binding to heme and heme crystals effectively kills malaria parasites. Screening an approved drug library for malaria or other diseases is potentially an efficient means to discover novel activities of existing drugs which can rapidly be translated into human clinical trials.

Some Uses of Metal-Based Complexes as Anti-Tumor Agents

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Background: Gold(III) complexes have long been known to exhibit anti-cancer properties. However, their medicinal application has been hampered by their poor stability in solutions. In this study, we aim at 1) preparing a variety type of physiologically stable gold(III) complexes; 2) studying the unprecedented structure-activity relationship for stable gold(III) complexes; 3) identifying potential anti-cancer drug hit(s) according to their in vitro/ in vivo anti-cancer and anti-angiogenic activities; and 4) illustrating their anti-cancer mechanism(s).
Methods: By using strong σ-donor dianionic porphyrinato ligands, we developed a family of gold(III) porphyrin complexes having different hydrophilic and hydrophobic substituents. Their IC_{50} values toward different panels of cancer cell lines were determined by MTT assay, and some of their in vivo anti-cancer activities were studied by using Buffalo rats and nude mice models. To elucidate the potent anti-cancer mechanism(s), DNA array, proteomics, western blotting and computational docking analyses have been employed.
Results: The IC_{50} values of the gold(III) porphyrin complexes were found to correlate with their lipophilicity and their cellular uptake. Two gold(III) porphyrins, namely gold-**1a** and gold-**2a**, exhibited tremendous in vitro cytotoxicity with IC_{50} ~ 50 nM toward some nasopharyngeal carcinoma cell lines. In vivo studies showed that these two complexes could prolong survival of the HCC-bearing rats and/or significantly inhibit tumor growth in nude mice models. Western blotting and computational docking analyses revealed that some anti-apoptotic proteins such as Bcl-2 and Mcl-1 are their important cellular targets. A gold(III) porphyrin with saccharide conjugation (gold-**3a**) was found to be relatively not cytotoxic, but exhibits significant cytotstatic and anti-angiogenic activities on MS1 cells.
Conclusions: The enhanced stabilization of the gold(III) ion and the ease of its structural modification render porphyrin ligands to be advantages in the development of physiologically stable bioactive gold(III) porphyrin complexes with potent anti-cancer and anti-angiogenic activities.

Personalized Medication with Estramustine Phosphate for Advanced Prostate Cancer after Screening of the CYP1A1 gene polymorphisms

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Background: Estramustine phosphate (EP) is a chemoendocrine agent applied for the treatment of advanced and/or hormone refractory prostate cancer. Gastrointestinal (GI) toxicity is observed frequently during EP therapy in prostate cancer patients. Formerly, we reported that polymorphisms in the CYP1A1 gene were significantly associated with GI toxicity during EP therapy. Aim of this study is to determine whether screening of the CYP1A1 gene polymorphisms is a useful method to achieve personalized medication with EP for advanced prostate cancer.
Methods: After screening of the CYP1A1 gene polymorphisms, a total of 39 patients with advanced or hormone refractory prostate cancer was regarded as a low-risk group for GI toxicity with EP. The methods of genotyping assay were a TaqMan assay or a direct-sequencing method. All of patients were administered EP 280 mg/day orally and assessed their toxicities monthly. Three of 39 patients withdrew EP therapy within 8 weeks because of other toxicities (liver dysfunction, lung edema, and gynecomastia), and they were excluded from the analysis. Formerly, we experienced EP therapy with same regimen for 55 patients of advanced prostate cancer without screening of the CYP1A1 gene polymorphisms. We compared the incidence of GI toxicity according to the NIH Common Terminology Criteria for Adverse Events v3.0 between former study and present one, and evaluated the efficacy of the gene screening. The Ethics Committee of the University of Tokyo approved this study.
Results: Follow-up period of present study was 557 ± 254.6 (mean± SD) days. Incidence of GI toxicity of former study and present one was 40.0% (22/55) and 19.4% (9/36), respectively. The odds ratio was 0.36 (95% CI, 0.13 – 0.94; P = 0.036). In the multivariate analysis, the hazard ratio was 0.21 (95% CI, 0.06 – 0.65; P = 0.006). The withdrawal of EP therapy because of GI toxicity in former study and present one was 18.2% (10/55) and 2.8% (1/36), respectively. **Conclusions:** Screening of the CYP1A1 gene polymorphisms prior to EP therapy could achieve a personalized medication with EP for the patients with advanced prostate cancer.

L-type calcium channels in non-excitable cells: critical roles in cell survival

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Background: Ca^{2+} is a highly versatile intracellular second messenger that regulates many complicated cellular processes, including cell functions, cell proliferation, and death. It has long been believed that in nonexcitable cells, including hematopoietic cells, store-operated Ca^{2+} entry is the principal route of Ca^{2+} influx. Increasing evidence suggests the existence of founctional L-type Ca^{2+} channels (LTCCs) in various immune cells, including T, B and natural killer cells, but their molecular entity and functions are poorly understood. We have previously reported that in mast cells IgE plus antigen (IgE/antigen) stimulates a dihydropyridine (DHP)-sensitive Ca^{2+} channel that is immunologically and pharmacologically related to LTCCs. In the present study we attempted to reveal the molecular entity of this Ca^{2+} channel. Moreover, since mast cells do not undergo apoptosis upon IgE receptor activation, while the Ca^{2+}-ATPase inhibitor thapsigargin (Tg), which cannot activate the LTCC-related Ca^{2+} channel, causes sizable apoptosis, we elucidated the potential role of the Ca^{2+} channel in mast cell survival.
Methods: The expression of LTCCs mRNA was evaluated using RT-PCR analysis. The expression of LTCCs on the cell surface was determined by flow cytometry. Apoptotic cell death and/or overall cell death were evaluated by annexin V/ propidium iodide double-staining, cytochrome c leakage and the membrane potential collapse.
Results: The mast cell line rat basophilic leukemia (RBL-2H3) expressed the α_{1C} subunit mRNA and express the α_{1C} protein on their surface. The cells underwent to apoptosis when extracellular Ca^{2+} was absent. The LTCC antagonists such as nifedipine and diltiazem remakably augmented IgE-mediated apoptosis, while the LTCC agonist (S)-BayK8644 rescued the cells from Tg-induced apoptosis. The augmentation and reduction of apoptosis were accompanied by the enhancement and suppression, respectively of mitochondrial Ca^{2+} efflux, depolarization, cytochrome c leakage, and caspase-3/7 activation. Finally, when the expression of the α_{1c} type of LTCC was knocked down using small interfering RNA, IgE/antigen alone caused substantial mitochondrial integrity disruption and apoptosis, as observed with IgE/antigen plus nifedipine or Tg stimulation, and (S)-BayK 8644 no longer protected cells from apoptosis.
Conclusions: LTCCs play a critical role in the survival of mast cells and potentially other immune cell types which express them through maintaning mitochondrial Ca^{2+} homeostasis and preventing mitochondrial integrity disruption.

Chemical chaperone therapy: magic bullet to the brain in G_{M1}-ggngliosidosis

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Background: Chemical chaperone therapy has been tried as a new molecular approach to brain damage in lysosomal diseases. Low molecular weight substrate analogues bind to mutant enzymes at neutral pH and dissociation occurs at acidic pH in the lysosome. Aims: 1) molecular analysis of chaperone-protein interaction in enzyme deficient cells. 2) assessment of chaperone effect in mice and humans.
Methods: Assays of *in vitro* enzyme inhibition and *in situ* chaperone effect of N-octyl-4-epi-□-valienamine (NOEV), using genetically engineered model mice expressing mutant human □-galactosdase. Computational modeling of the human □-galactosidase structure and theoretical calculation of NOEV-enzyme interaction, leading to the chaperone effect. DNA microarray on secondary gene expression. Analysis of neurotrophin receptor Trk using specific antibodies. NOEV effect on intracellular signal transduction, autophagy, and gene expression.
Results: NOEV was clinically effective within 3 month when oral administration was started at 2 month of age ($p<0.05$ at 5-7 months; n=11-15 for non-treated and n=7-16 for treated mice). The effect was not evident till 6 month of treatment (11 month of age) when treatment was started at 5 month of age ($p<0.05$ at 11 months; n=5 for non-treated and n=6 for treated mice). Computational prediction showed that human □-galactosidase took the TIM barrel structure, and the NOEV-enzyme binding was less strong in the lysosomal environment at low pH, resulting in dissociation of the complex. DNA microarray revealed an abnormal pattern of gene expression in enzyme-deficient human and mouse fibroblasts, and mouse brain. Phosphorylated Trk and Bip/GRP-78 abnormally increased at the late clinical stage of G_{M1}-gangliosidosis in the mouse brain. These pathological changes were corrected by NOEV treatment.
Conclusions: Early chaperone thrapy is mandatory for better neurological effect in model mice. Computational modeling is useful for analysis of molecular pathology in G_{M1}-gangliosidosis. Multiphasic intracellular abnormalities were corrected by NOEV threrapy in association with the clinical effect. These new approaches are important for understanding chemical pathology and developing new therapeutic trials in lysosomal diseases.

Antimalarial drug resistance in Indonesia: A molecular analysis

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Background: Malaria remains a major public health problem in Indonesia, causing approximately 30 million clinical cases and several thousands deaths annually. Since the first report of P. falciparum resistance to chloroquine (CQ), the first-line antimalarial, in the mid of 1970s in East Kalimantan and West Papua Provinces, respectively, resistance to this drug has rapidly spread throughout the archipelago. Resistance to CQ have also been reported in P. vivax and P. malaria in 1996 and 2003 respectively. Sulphadoxine-Pyrimethamine (SP) resistance was first reported in the early 1980s in West Papua and then sporadically found in other islands of Indonesia. The Ministry of Health has adopted the policy to use artemisinin-based combination therapy (ACT) as the first line antimalarial drug in the year 2004. To determine the extent of antimalarial drug resistance among the field isolates throughout the archipelago, a molecular epidemiologic survey has been conducted since 1998
Methods: Molecular assays of the parasite DNAs isolated from blood blots on filter paper, collected from several sentinel sites, were performed. The assays employed Polymerase Chain Reaction (PCR), restriction fragment length polymorphism (RFLP) to detect the allelic forms of the pfcrt and pfmdr1 genes, and pfdhfr and pfdhps genes and their orthologues in P. vivax, that were associated with CQ and SP resistance, respectively.
Results: The results revealed that the pfcrt 76-Thr and pfmdr1 86-Tyr alleles have been fixed in many of (VH1) P. falciparum isolates examined. Analysis of dhfr gene revealed three mutant alleles 16Val, 59Arg and 108Asn/Thr. The dhfr 108Asn mutations appeared either as a single mutation or paired with 59-Arg in the frequency of 20-90% among the isolates examined. Mutant alleles of the dhps gene appeared in much less frequency, mostly in the form of 437G and 540K alleles. Analysis of the P. vivax isolates did not find any mutations in the pvcg10 and pvmdr1 genes, but found several isolates that carried mutant alleles of the pvdhfr and pvdhps genes. The findings strongly indicate resistance to chloroquine in P. falciparum isolates and a growing resistance status to sulfadoxine-pyrimethamine combination in both P.falciparum and P. vivax.
Conclusions: molecular evidence indicates that the antimalarial drug resistance in Indonesia poses a continuing challenge to the malaria control programme and highlights the need to the proper antimalarial deployment.

Anti-apoptotic and apoptotic action of (-)deprenyl (Selegiline) and its metabolites

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Background: The antiapoptotic effect of (-)deprenyl on human phaeochromocytoma cells after serum deprivation has been reported earlier.
Material and Methods: In our experiments the mode of cytoprotective action of (-) deprenyl was studied using A2058 human melanoma cells in culture. Serum deprivation for five days resulted in excessive number of apoptosis in the cell ciltures.
Results: Very low doses of (-) deprenyl (10(-7)-10(-13)M) caused an approximately 2 days delay in the onset if apoptosis. The known metabolites of (-)deprenyl, (-)desmethyl-deprenyl, (-) and (+) methylamphetamine and also (+) deprenyl failed to exert the same effect. The anti-apoptotic action of (-)deprenyl was prevented by the simultaneous application of the microsomal drug-metabolizing enzyme inhibitor SKF-525A, showing that (-) deprenyl needs metabolic conversion in order to be anti-apoptotic, but the effective metabolite is still unknown. On the other hand, higher dose (10(-3Ml) of (-) deprenyl, (-) desmethyl-deprenyl, (-) and (+) methylamphetamine induced apoptosis in the non-serum deprived A-2058 cell culture. SKF 525A did not prevent the apoptosis-inducing effect of (-) deprenyl which means that no metabolic changes are needed for this activity. High dose (10(-3) Ml) (-)deprenyl induced very high Caspase 3 activity in non-serum-deprived A-2058 cell culture, low doses (10(-9)-10(-13)M maintained Caspase 3 activity on control level in case of serum deprivation.
Conclusion: The neuroprotective effect of (-) deprenyl (Selegiline) may be due to the anti-apoptotic activity of this compound.

Advent of Pharmacology of Nociceptors Initiated by Capsaicin Has Opened Also a New World in Neurohumoral Regulation

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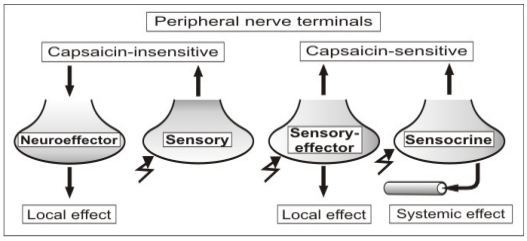
Background: Capsaicin, the hot principle of chilli is the first magic bullet for nociceptors. Its receptor cloned in 1997 is a thermosensor ion channel gated by noxious stimuli, named Transient Receptor Potential Vanilloid 1 (TRPV1). Furthermore, substance P and other neuropeptides released from the activated capsaicin-sensitive nerve endings elicit – without axon reflexes – neurogenic inflammation and other efferent tissue responses. We also described that released somatostatin inhibits inflammation at distant sites.
Methods: 1. Mechanical, noxious heat thresholds of rat paw was measured in hyperalgesia (plantar incision, heat injury etc.) with and without stimulation of the acutely denervated contralateral paw. 2. Neurogenic inflammation evoked by electrical stimulation (dorsal roots, nerves) or by irritants was similarly tested after stimulating other parts of the body (paw, eye, vagal nerve). 3. Sensory neuropeptides were measured by radioimmunoassay. 4. Gene deleted mice (TRPV1, sst4R) were also used.
Results: 1. Contralateral counter-irritation (capsaicin, mustard oil) diminished the hyperalgesia by more than 50%. Antagonists of somatostatin (cyclosomatostatin) or cannabinoid CB_1 receptor counteracted these effects. 2. Antidromic stimulation (dorsal root, sciatic nerve) enhanced the plasma somatostatin level over 3-fold and inhibited by around 50% inflammation evoked by capsaicin, carrageenin or contralateral dorsal root stimulation. The inhibition was absent in rats pretreated with somatostatin antibody or after perineural capsaicin pretreatment. 3. 0.1 Hz electrical stimulation did not evoke neurogenic inflammation but still produced systemic inhibitory effects.
Conclusions: Capsaicin-sensitive major subsets of sensory neurons subserve unorthodox local effector and systemic antinociceptive, antiinflammatory „sensocrine" functions. Drug candidates of TRPV1 antagonists or somatostatin receptor agonists are in Phase II clinical trials.



Treating the Untreatable: Alpha 1-Adrenoreceptor Antagonist Prazosin for PTSD, Disruptive Agitation in AD and Alcohol Dependence

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Background: Although the CNS noradrenergic system has been implicated in multiple neuropsychiatric disorders, the postsynaptic alpha 1-adrenoreceptor (alpha1-AR) has been addressed as a pharmacotherapeutic target only in cardiovascular and urologic disorders. Clinical and neuropathologic studies suggest that increased responsiveness to norepineprhine (NE) at the alpha1-AR contributes to the pathophysiology of post-traumatic stress disorder (PTSD) trauma nightmares, disruptive agitation in Alzheimer's disease (AD) and alcohol dependence. Prazosin, a CNS active alpha1-AR antagonist, was evaluated for efficacy and tolerability in these three difficult to treat disorders.
Methods: Study 1: 40 Vietnam war veterans with PTSD and intractable nightmares were randomized to prazosin (achieved mean bedtime dose = 13 mg) or placebo. 3 weeks of titration and 8 weeks of mean dose. Study 2: 22 elderly AD patients with treatment resistant disruptive agitation were randomized to titrated prazosin (achieved daily dose 2 mg id and 3 mg bedtime) or placebo. 2 weeks of titration and 4 weeks of mean dose. Study 3: 17 men seeking abstinence for alcohol dependence were randomized to titrated prazosin (achieved dose 4 mg bid and 8 mg bedtime). 2 weeks of titration and 4 weeks of mean dose.
Results: Prazosin was well tolerated in all studies. Prazosin was significantly and substantially superior to placebo for: (study 1) reducing nightmares and improving sleep and overall clinical status in PTSD; (study 2) reducing disruptive agitation by Neuropsychiatric Index (NPI) and Brief Psychiatric Rating Scale (BPRS) scores in AD; and (study 3) reducing alcohol ingestion and days drinking in persons seeking abstinence.
Conclusion: Prazosin appears effective for three different neuropsychiatric disorders that have been very difficult to treat. Larger and longer placebo controlled trials of prazosin are underway for all three conditions. This data introduces the novel use of prazosin, an alpha1-AR antagonist, for several different neuropsychiatric disorders that have been very difficult to treat.

Role of estradiol and testosterone in the regulation of bone metabolism in men and women

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Sex steroids (testosterone, 17□-estradiol) are major regulators of bone metabolism in men and women. After menopause, dramatic decrease in the secretion of 17□-estradiol results in an increase in bone resorption followed (but not matched!!) by increased bone formation. This imbalance between bone formation and bone resorption results in a decrease in bone mineral density (BMD) and an increase in the risk of fracture. Structural mechanisms underlying postmenopausal bone loss have been partly elucidated (trabecular perforation and loss, loss of trabecular connectivity, cortical thinning due to, mainly but not only, higher endocortical resorption and trabecularization of subendocortical bone).
In men, the major sex steroid is testosterone, however, 17□-estradiol is probably the principal regulator of bone metabolism in men. During growth, 17□-estradiol is necessary for closure of growth cartilages and growth arrest. Data are discordant as concerns the role of sex steroids in the regulation of the radial bone growth in boys. During ageing, lower concentration of 17□-estradiol, especially of its bioavailable fraction, is associated with higher levels of biochemical bone turnover markers, lower BMD, slightly faster bone loss. Direct effect of testosterone on bone formation and bone resorption in men is probably weak. Less data concern the role of both sex steroids in the regulation of metabolism of trabecular bone and cortical bone in male and they are based largely on experimental studies. Trabecular bone seems to be more sensitive to lower 17□-estradiol concentration, because its metabolically available area is higher. It is not clear if the sex steroids play a significant role in the regulation of periosteal apposition in older men. Deficits of both hormones increase the risk of fracture in older men; 17□-estradiol deficit acts probably mainly through the effect on bone (BMD, microarchitecture) and testosterone deficit acts at least partly through its effect on muscle mass and risk of falls.
In both sexes, the 17□-estradiol deficit may interact with other factors influencing bone metabolism. Smoking may aggravate consequences of 17□-estradiol deficit through the influence on its synthesis and catabolism. Conversely, obesity can partly counteract the effect of 17□-estradiol deficit, partly by the peripheral aromatization and partly by the mechanical effect.

Effects of neurotoxic and neuroprotective compounds on cholinergic neurons are mediated by alterations in acetyl-CoA metabolism

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Background: The preferential loss of septal cholinergic neurons is a main cause of cognitive deficits in course of Alzheimer's disease (AD) and other encephalopathies of advanced age. High susceptibility of cholinergic neurons to neurodegeneration might result from the fact that they utilize acetyl-CoA, not only for energy production but also for acetylcholine (ACh) synthesis. Therefore, acetyl-CoA metabolism is a likely target for both cholinotoxic insults as well as for therapeutic approaches.
Methods: Cholinergic septum-derived SN56 neuroblastoma cells were cultured in Dulbecco-Eagle's medium for 2 days with subsequent treatment with neurotoxic or/and cytoprotective compounds.
Results: The differentiation of SN56 cells by retinoids, NGF or cAMP-mediated signals, caused the increase of choline acetyltransferase (ChAT) activity, ACh synthesis/quantal release and cytoplasmic acetyl-CoA content along with decrease in acetyl-CoA synthesis and its levels in neuronal mitochondria. The shortage of acetyl-CoA in mitochondria of differentiated cells caused their greater than nondifferentiated ones susceptibility to recognized AD pathogens such as amyloid-beta, NO, Al and Zn. These compounds caused dose-dependent increase of nonviable cell fraction and cytoplasmic cytochrome c levels, decreases in mitochondrial enzyme and ChAT activities, intramitochondrial and cytoplasmic acetyl-CoA and ACh levels, with loss of morphologic differentiation. Number of nonviable cells inversely correlated with pyruvate dehydrogenase activity (r=-0.79, p=0.002) and content of mitochondrial acetyl-CoA (r=-0.92, p=0.0002). Neuroprotective capacity of lipoates correlated with their ability to maintain high level of acetyl-CoA in mitochondria. On the other hand, the expression of cholinergcic phenotype positively correlated with alterations in cytoplasmic acetyl-CoA levels (r=0.90, p=0.002).
Conclusions: These data indicate the existence in cholinergic neurons two functionally independent pools of mitochondrial and cytoplasmic acetyl-CoA, that under pathologic conditions affect their viability and expression of cholinergic phenotype, respectively.
Authors' disclosure statement: Supported by MNISW projects 2P05A 110 30.

Titanium-Based Magic Bullets Against Renal-Cell Cancer

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Background: Titanocene dichloride (Cp_2TiCl_2) shows significant activity against a variety of human cancer cell lines, overcomes the resistance against platinum drugs and reached two clinical Phase II studies against renal cell and metastatic breast cancer, where Cp_2TiCl_2 failed due to a lack in anti-proliferative activity.
The aim of our research is to synthesise suitably substituted titanocenes and ansa-titanocenes in order to control the physiological uptake of the titanocenes and to overcome the toxicity effects exhibited by titanocene dichloride. In addition we model the titanocene-DNA interaction leading finally to apoptosis.
Methods: Ansa-titanocene dichloride derivatives are obtained through reductive dimerisation of substituted aryl fulvenes with titanium dichloride, while un-bridged titanocene compounds are available from fulvenes through the addition of lithium hydride or aryl lithium [Bioorganometallic Fulvene-Derived Titanocene Anticancer Drugs, K. Strohfeldt, M. Tacke, Chem. Soc. Rev., **2008,** 37, 1174-1187].
Results: We investigate the cytotoxic activity of all titanocenes on LLC-PK pig kidney carcinoma cells and that of selected titanocenes on a 36 cell-line panel in vitro. Results show that our most effective titanocene has a significantly higher activity than Cp_2TiCl_2 itself. Recently, our best titanocenes have successfully finished pre-clinical animal studies (EAT, MCF7, PC3, A431 and CAKI-1 in mice) as well as clinical ex vivo tests involving a variety of explanted human tumors.
Conclusions: Our best titanocene derivatives are ready for clinical trials and have the potential to become the first effective chemotherapy against advanced renal-cell cancer in the nearby future.
Acknowledgement: Support is acknowledged from CESAR, COST, HEA/CSCB and UCD.

Chemotherapeutic agents for Merkel cell carcinoma (MCC) of skin – past, present and future

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Background: In the past, chemotherapy was mostly used for metastatic MCC, although a few authors tried adjuvant chemotherapy as well. At the present time, multi-modality treatments are used. This study reports the multicenter experience on management of MCC.
Methods: Patient information was obtained from medical records of 3 Canadian and 1 French institutes. Cox-proportional hazard analysis was used to find significant prognostic factors for cause-specific (CSS) and overall survivals (OS).
Results: From 1987 to 2007, 121 cases from 4 institutes were collected. Median follow-up was 21.5 (range:0.5–163.4) months. The overall rate of distant metastases was 36/121 (30%). A total of 25 patients received chemotherapy either as initial treatment (n=10 or later on at time of relapse (n=15). Patients were treated with multiple regimens at recurrence. Chemotherapeutic agents included: Etoposide (E), Cisplatin (P), Carboplatin, Cyclophosphamide (C), Adriamycin (A), Vincristine (V), Epirubicin (Epi), Irinotecan, Taxotere and Topotecan. The regimens were: EP (13 patients), CAV/EP (6 patients), CAV or CEpiV (3 patients), AV/E or CEpiV/E (2 patients), CEP (1 patient), weekly carboplatin (1 patient), irinotecan+carboplatin (1 patient), taxotere and later topotecan (1 patient). The latter were used as third and fourth line chemotherapy in the same patient, with no response. Irinotecan+carboplatin produced a partial response. The patient who received weekly carboplatin as a radiosensitizer had a partial response. Cox-proportional hazard analysis showed that postoperative adjuvant radiotherapy and stage were significant prognostic factors for CSS and OS.
Conclusions: The future magic bullet for MCC will likely be radiotherapy in combination with modern chemotherapy. Future studies of MCC should focus on newer agents used for other neuroendocrine tumors, e.g., irinotecan, taxotere and topotecan.

Assay of Therapeutic Effect in Hepatitis Using Pharmacokinetics of Salicylamide: Effects of Sho-saiko-to, a traditional Kampo medicine, and Its Chemical Components in Carbon Tetrachloride Intoxicated Rats

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Background: Pharmacokinetics (PK) is a powerful tool for detecting physiological changes in vivo resulting from injury, tumors or pregnancy. Aims: The therapeutic effects of Sho-saiko-to and its chemical components, baicalin, baicalein, glycyrrhizin and glycyrrhetic acid, were examined using daily changes in PK behavior of salicylamide (SAM) in rats with carbon tetrachloride (CCl_4)-induced hepatitis.
Methods: Male Wistar rats were intraperitoneally (i.p.) injected with 0.2 ml/kg of CCl_4 in 20% olive oil, and serum ALT value measured daily. A suspension of Sho-saiko-to extract was given orally at 40 mg/kg one hour before CCl_4 dose; while each component solution was given i.p. at the same dose. The rats were treated daily with the reagents in the same manner. SAM was injected intravenously into CCl_4-intoxicated rats at days 0 to 5 and blood samples collected for 90 min. SAM in blood samples was determined by its fluorescence. The PK of SAM were examined using the parameters; plasma clearance (CL), the mean residence time (MRT), the volume of distribution (Vd_{ss}) at steady state, etc.
Results: Serum ALT value rose to a peak one day after CCl_4 dose and then decreased to the control level after three days. Baicalin (25.3%) and glycyrrhizin (19.9%) effectively suppressed the peak value of CCl_4-induced hepatitis, but Sho-saiko-to extract (39.1%), baicalein (36.4%) and glycyrrhetic acid (47.1%) were less protective. However, the PK of SAM showed that at day one the primary decrease of CL resulted in liver damage because of CCl_4 intoxication, in which the metabolic enzymes were damaged but the damaged tissue remained. Thus there was delayed MRT and Vd_{ss} remained at a control level. Furthermore, the secondary decrease of CL at day three may result in liver regeneration where the active enzymes were eliminated and the damaged tissue was removed. That is, MRT remained at a high level and Vd_{ss} decreased. Sho-saiko-to extract or its components protected the liver from damage in the different ways, as seen in the PK parameters; e.g. baicalin kept MRT at a control level but glycyrrhizin decreased it.
Conclusions: The PK for SAM showed that liver function recovered in a biphasic manner by five days after CCl_4 intoxication. Sho-saiko-to extract or its components effectively protected the liver against damage.

Telephone counseling of athletes abusing anabolic-androgenic steroids and the state of drugabuse in Japanese athletes

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Background: Drug abuse, most notably anabolic-androgenic steroid (AAS) use, in athletes is widespread. As a result, athletes and exercise enthusiasts who abuse these drugs are troubled by the side effects of these illicit drugs, especially AAS.
Methods: In an attempt to improve this situation, since 1993, we have counseled athletes who abuse drugs and others with questions about AAS via telephone and tabulated the results. Counseling sessions took place by telephone every Monday between 19:00-23:00 hours. The number of cases was tabulated each year and the specific items discussed during each consultation were categorized by key words. Cases consisted of both drug abusers and athletes who did not abuse drugs and were concerned about the side effects or other various problems surrounding the use of AAS.
Results: From 1993 to 1996 there were about 50 cases yearly; thereafter, the number of consultations dropped to about 30 to 40 cases each year. In 2002, consultations with drug abusers accounted for 52.2% of all consultations compared with 46% of all consultations from 1993 to 2002. We have found that abusers of endocrine agents exist in Japan, as well as elsewhere.
Conclusions: 1) We hope these results will demonstrate the necessity of employing public institutional counseling systems for athletes who are drug abusers in Japan, similar to the successful system instituted by the Swedish National Service. 2) Furthermore some athletes and exercise enthusiasts has been recently using the website that drugabusers control in Japan. They exchange the information about the knowledge of drugabuse each other. We accessed to the website and analyzed the contents of them by key words. We would like to report the result in detail, too

Interferon Gene Therapy against Metastatic Cancer

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Background: The interferon (IFN) is the family of one of the most potent cytokines with various biological activities. Both type I (IFN-arfa and beta) and type II (IFN-gamma) IFNs have been used as therapeutic agents for malignant tumors and hepatitis in clinic. However, the biological half-lives of IFNs are very short. One of the promising approaches to overcome this drawback may be IFN gene therapy.
Methods: The therapeutic effect of IFNs was examined using mouse models of liver or lung metastasis following gene transfer by intravenous injection of plasmid DNA vector by the hydrodynamics-based procedure (1). In order to prolong the duration of gene expression, we designed CpG-reduced (about 80%) or completely depleted vector encoding IFNs. Therapeutic effect on allergic diseases was also evaluated using NC/Nga mice, a model for human atopic dermatitis.
Results: A significant amount of IFNs was observed in the liver and blood circulation following gene expression. In the liver metastasis experiment, IFN-expressing vector showed a profound reduction of liver metastasis and a prolonged survival (2). Hydrodynamic delivery of CpG-reduced vectors resulted in more sustained production of IFNs and a better therapeutic effect against the lung metastasis (3). Moreover, a single hydrodynamic injection of completely CpG depleted vector resulted in much more prolonged concentration of IFN-gamma over 80 days in NC/Nga mice. The correction of the imbalance of helper T lymphocyte subpopulations was shown in the mice and various symptoms associated with the allergic disease were eliminated.
Conclusions: 1) Novel CpG-reduced vectors encoding IFNs showed prolonged gene expression compared with CpG-rich conventional vectors following intravenous injection by the hydrodynamic method. 2) Sustained IFN gene expression by CpG-reduced vectors resulted in enhanced therapeutic effect in lung metastatic model in mice. 3) Prolonged plasma IFN concentration was also effective for the treatment of atopic dermatitis in mice. In conclusion, IFN gene therapy would be possible through optimization of design and delivery of IFN plasmid vector.

References:

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Interaction Between Local Anesthetics, Specially Articaine, and the Sarcoplasmic Reticulum Ca^{2+}-Adenosine Triphosphatase

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Background: The calcium-dependent adenosine triphosphatase (Ca^{2+}-ATPase) is a major intrinsic protein in the sarcoplasmic reticulum (SR) membranes from skeletal muscles. It actively transports Ca^{2+} from the cytoplasm to the SR lumen, reducing the cytoplasmic Ca^{2+} concentration to promote muscle relaxation. Local anesthetic (LA) may induce sustained muscle contraction by inhibiting Ca^{2+}-ATPase. Aims: 1) to determine the action mechanism of articaine on the Ca^{2+}-ATPase cycle, 2) to study the different LA sensitivities on skeletal muscle isoforms of the Ca^{2+}-ATPase.
Methods: SR vesicles from rabbit skeletal muscle were obtained as in Champeil et al (1985). Ca-dependent ATPase activity was determined as in Baginski et al (1967). ATP-dependent calcium uptake and phosphorylation with inorganic phosphate (P_i) were determined with a radioisotopic technique. Transient kinetics of the Ca^{2+}-ATPase active transport was analyzed by numerical simulation (Hecht et al, 1990).
Results: Articaine inhibited Ca^{2+}-ATase activity, with Ki depending on Ca^{2+} concentration: it increased from approximately 6 mM for 0.1 μM Ca^{2+} up to a constant value around 40 mM at $[Ca^{2+}]$ higher than 20 μM. Anesthetic also inhibited the Ca^{2+} uptake by isolated SR vesicles (Ki=30.53±3.4mM, n=5). Articaine increased the permeability of the membrane to Ca^{2+} and was prevented by Ca^{2+} and Mg^{2+}. Ca^{2+}, Mg^{2+} and H^+ affected the inhibitory action of articaine on the Ca pump protein. In addition, we studied the properties and inhibitory effect of several LA (unplublished results) on Ca^{2+}-ATPase from masticatory muscles (K_i(lidocaine)= 19.31±1.87, n= 6 for ATPase activity, K_i(lidocaine)= 25.10±2.95,n=5 for Ca^{2+} uptake. K_i(bupivacaine)= 19.30±1.12, n= 5 for Ca^{2+}-ATase activity and K_i(bupivacaine)= 8.12±2.81, n=5 for Ca^{2+} uptake).
Conclusions: 1) The activating effect of Ca^{2+} on the ATPase activity was competitively inhibited by articaine. 2) The activating effect of Mg^{2+} on the phosphorylation of Ca^{2+}-ATPase by P_i was also inhibited by articaine. 3) Decreasing pH increased K_i for articaine to inhibit the Ca^{2+}-ATase activity. 4) Articaine did not affect the reaction mechanism of the cations acting as cofactors of ATP in the catalytic site. 5) K_i values for lidocaine and bupivacaine in masticatory muscles were lower than those reported for these LA in white fast muscle.

Paclitaxel works nicely with molecular targeting cancer therapy via RNA interference

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BACKGROUND: Paclitaxel (PTX; Taxol®) is used to treat patients with lung, ovarian, breast, head, and neck cancer or advanced Kaposi's sarcoma. PTX works by interfering with normal microtubule breakdown during cell division. Although it acts as a potent anti-cancer reagent, it has a major dose-limiting toxicity in that it causes bone marrow suppression, especially neutropenia. To reduce the dosage of PTX, and to minimize hematologic side effects, we designed a combination therapy based on molecular targeting (in which the target is VEGF) via RNA interference (RNAi). VEGF (vascular endothelial growth factor) is a potent angiogenic factor in cancers.
METHODS AND RESULTS: We established a human VEGF blockade system via RNAi. The synthetic short interfering RNA (siRNA) targeting human VEGF almost completely inhibited VEGF secretion by a human prostate cancer cell line (PC-3). The VEGF siRNA, together with atelocollagen, significantly suppressed the growth of PC-3 tumors that had been subcutaneously xenografted in nude mice. Atelocollagen, which is derived from bovine collagen digested with pepsin to reduce the antigenicity, is used as a delivery carrier of siRNAs. Atelocollagen contributes to the stabilization of the siRNA in tumors and delivers the siRNA to tumor tissues. These inhibitory effects of VEGF siRNA were dramatically augmented by combined treatment with PTX. The combinatory effect was cytocidal, thus reducing the initial volume of the tumor. The PTX dose (12 mg/kg) used for the combinatorial treatment did not cause any neutropenia or liver damage. VEGF siRNA suppressed VEGF contents in tumors, leading to the inhibition of tumor angiogenesis. Surprisingly, we also observed, for the first time, that PTX itself significantly reduced VEGF expression in the tumor. The evidence supports the phenomenon that PTX inhibits tumor angiogenesis even without any evidence of the molecules involved.
DISCUSSION AND CONCLUSION: We made it possible to reduce the dosage of PTX, a chemotherapeutic agent, in combination with a molecular targeting therapy consisting of nucleic acids, especially those based on RNAi. We also found that PTX works nicely in combination with molecular targeting cancer therapy, i.e., anti-angiogenesis (ant-VEGF), via RNAi.

Crosstalk Between TNF□ and NGF: Potential Implications for Alzheimer's Disease and Neuroblastoma

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Background: Neuroblastoma is a pediatric tumour of the sympathetic nervous system. While early stage neuroblastoma sometimes shows spontaneous regression, advanced neuroblastoma has poor outcome and new therapeutic strategies are needed. Nerve growth factor (NGF) can induce differentiation of neuroblastoma cells expressing high levels of an NGF-specific receptor $p140^{TrkA}$. However, many neuroblastoma cells in advanced stages express $p140^{TrkA}$ poorly and they cannot differentiate in response to NGF, though overexpression of $p140^{TrkA}$ allows NGF to induce their differentiation. Thus, inadequate expression of $p140^{TrkA}$ is supposed to cause the insensitivity of neuroblastoma cells to NGF-dependent differentiation.
Results: we have reported recently that overexpression of $p140^{TrkA}$ is not necessary for NGF-dependent differentiation of neuroblastoma cells. NGF can induce synthesis of tumour necrosis factor □ (TNF□) in neuroblastoma cells. Although TNF□ is a cytokine that can promote cell death through TNFα receptor 1, TNF□ induced by NGF activates another TNFα receptor, TNFR2. When signalling through TNFR2 was inhibited, NGF induced differentiation of neuroblastoma cells without overexpression of $p140^{TrkA}$. Thus, signalling through TNFR2 appears to cause the insensitivity of neuroblastoma cells to NGF-dependent differentiation, rather than insufficient expression of $p140^{TrkA}$.
Conclusion: Our findings could provide new therapeutic strategies for neuroblastomas. Furthermore, induction of TNF□ in neural cells by NGF suggests a positive feedback loop of TNF□ and NGF expression between neurons and glial cells, since glial cells can produce NGF in response to TNF□. This loop could promote survival of neural cells in inflammation. However, once their balance is disturbed, this loop could contribute to either excessive death of neural cells or excessive proliferation of immature neural cells, i.e. neurodegenerative or cancerous conditions.

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Mechanism of Fluoroquinolone resistance in *Shigella* and *Salmonella* species

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Background: Enteric fever and shigellosis remain a major cause of morbidity and mortality in worldwide. Ciprofloxacin, a fluoroquinolone antimicrobial agent is generally highly effective in treating typhoid fever and shigellosis patients. We report the emergence of complete ciprofloxacin resistance in Bangladesh.
Objectives: The aim of the present study was to characterize and understand the molecular mechanism of fluoroquinolone resistance in Shigella flexneri and Salmonella Typhi strains recently isolated in Bangladesh.
Methods: A total of 1728 Shigella species isolated between 2004 and 2008 and 765 Salmonella Typhi strains isolated between 2006 and 2007 in the Clinical Microbiology Laboratory from diarrhoeal patients at ICDDR,B hospital were tested for MIC by E-test and agar dilution method in addition to antibiotic susceptibility testing following the recommendations of CLSI. Plasmid profiling, PFGE and sequencing analysis were performed in order to determine the clonal relationships and mutation analysis of QRDR of gyrA, gyrB and parC.
Results: Of 1728 Shigella species isolated between 2004 and 2008, 104 (6%) isolates were resistance to ciprofloxacin. Most of ciprofloxacin resistant isolates were S. flexneri 2a (90%). Resistance to ciprofloxacin of S. flexneri 2a increased from 0% in 2004 to 95% in June 2008. The MIC for ciprofloxacin, norfloxacin, ofloxacin, were 6-8 µg/ml, 8-32 µg/ml, and 8-24 µg/ml, respectively. Of 765 Salmonella Typhi strains, 474 (62%) strains were resistant to nalidixic acid. Of the 474 nalidixic acid resistance strains, 402 (85%) showed reduced susceptibility to ciprofloxacin. The complete resistance to ciprofloxacin increased from 18 (2.8%) in 2006 to 11 (4.5%) in July 2007. The isolates showed reduced susceptibility to ciprofloxacin had the MIC ranged from 0.064-0.25 µg/ml and the complete ciprofloxacin resistance strain showed the MIC ranged from 6-32 µg/ml. Sequence analysis of QRDR of ciprofloxacin resistant strains of S. flexneri and S. Typhi revealed that all had mutations in gyrA ($Ser^{83} \rightarrow$ Phe) and/or ($Asp^{87} \rightarrow$ Asn or Gly) and a single mutation in parC ($Ser^{80} \rightarrow$ Ile) whereas none of the susceptible strain had the mutation in their QRDR region. In addition, a novel mutation point was detected at outside the QRDR at position 211 (His→ Tyr) in gyrA gene of fluoroquinolone resistant strains of S. flexneri 2a. None of the strains of S. flexneri 2a and S. Typhi had mutations in gyrB and parE genes. Qnr gene was not detected in any of strains which exclude the possibility of plasmid mediated quinolone resistance in S. flexneri and S. Typhi in this region. A single PFGE type A, subdivided into four subtypes A1 to A4, were found in S. flexneri 2a resistant strains suggesting their genetic relatedness. Similar type of findings was observed in case S. Typhi 2a resistant strains.
Conclusions: The present study reported the mutation in fluroquinolone resistant S. flexneri 2a and S. Typhi strains in Bangladesh. Increase isolation of ciprofloxacin resistance strains suggests that S. flexneri and S. Typhi are becoming difficult to treat in Bangladesh.

Analysis of fish immune response through bacterial stimulation and its application to oral vaccination for the cultured marine fishes

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Background: The establishment of systems to display heterologous proteins on the cell surface of microorganisms is expected to be useful in the separation of produced polypeptides; the production of microbial biocatalysts, whole-cell adsorbents, and live vaccines; and the screening of modified or novel proteins. Thus, since cell-surface display is a promising approach in protein engineering, by using the cell-surface engineering system in S. cerevisiae, we tried to express the 380R antigen from red sea bream iridovirus (RSIV), which is one of famous viral diseases in the cultured marine fish.
Methods: We have cloned the 380R antigen from RSIV and purified the recombinant 380R from Escherichia coli. 200ul of the purified recombinant 380R antigen (approx. 0.8mg/ml) was injected and the antiserum was collected from two rabbits. Western-blot analysis revealed that anti-380R antigen antibody immunoreacted with the recombinant proteins in E. coli. Next, we tried to detect the expression of the recombinant protein on the outside of the yeast cells by using the antibody.
Results: The recombinant 380R antigen on the yeast cells was detected by immunofluorescence labeling methods. Finally, we tried to use whether the display system of arming yeast could be applied for oral vaccination in RSIV. RSIV infection test towards Oplegnathus fasciatus clearly indicated that the arming yeast carrying the recombinant 380R antigen immediately decreased mortality rate.
Conclusions: Further work is needed to analyze the immunological system in fish and to show whether the arming yeast carrying the candidate for target antigens from RSIV and how oral vaccination works in the red sea bream. Therefore, we have used the zebrafish, an ideal model for developmental research that has now emerged as a valuable tool for immunological study.

Targeted Delivery of Magic Bullets by the Use of Mechanized Nanoparticles

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Background: Recent development in Nanotechnology led to the generation of a variety of nanomaterials including nanoparticles, nanosheets, nanofibers and nanotubes. Nanoparticles with diameters in the range of 100-200 nm are of particular interest, as they can serve as efficient vehicles to store and deliver magic bullets. In addition, nanoparticles provide protection of stored drugs. Targeting ligands can be attached to the nanoparticles to achieve cancer targeting. Finally, nanomachines can be attached to accomplish controlled release of magic bullets. We are developing mesoporous silica nanoparticles to achieve delivery of anticancer drugs.
Methods: Camptothecin was chosen to test the ability of mesoporous silica nanoparticles to deliver poorly water soluble anticancer drugs to cancer cells. The nanoparticles were fluorescently labeled to follow their cellular localization. Camptothecin was loaded onto the nanoparticles, added to human cancer cells and cellular effects were investigated by examining apoptosis induction caused by camptothecin. In the second experiment, we coated the pore interior with azobenzene to generate nanoimpellers that release the stored drug in response to light exposure. Operation of nanoimpellers was examined by the release of a dye, propidium iodide, and following staining of nuclei in response to light exposure. Light dependent release of anticancer drugs using nanoimpeller was examined by using camptothecin.
Results: Camptothecin was efficiently stored in fluorescent mesoporous silica nanoparticles and was released in human cancer cells as detected by the induction of apoptosis. Uptake of the nanoparticles and their lysosomal localization were established. Attaching targeting moiety on the surface of nanoparticles enabled preferential effects on cancer cells. Nanoimpeller was shown to operate in human cancer cells.
Conclusions: Mesoporous silica nanoparticles provide promising vehicles to deliver anticancer drugs. This is particularly important, as a significant percentage of anticancer drugs being developed is poorly water soluble and has problems in their clinical use. Nanoimpellers provide vehicles that can accomplish controlled release of anticancer drugs in response to light exposure. Our studies open up new approaches to deliver magic bullets.

Carbapenem –resistant Bacteroides isolated in Japan

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Background: Bacteroides fragilis group are one of the most clinically important group among anaerobic bacteria. Related infections are usually mixed infection with several anaerobic and aerobic bacteria. Most of the isolates in B.fragilis group produce ß-lactamase with a variety spectrum of substrate specificity and it may affect activity of the ß-lactams used. Besides ß-lactam resistant, B.fragilis group shows resistance to wide range of antimicrobial agents. On the other hand, initial chemotherapy for anaerobic infection is necessarily empiric because it takes time to define isolated anaerobes and their susceptibility. Because of the wide range of resistance in B.fragilis group, useful agents for empiric therapy are restricted. Carbapenem(Carb)s are useful and important agents for anaerobic infection. We studied on Carb resistant strains in B.fragilis group.
Methods: Susceptibility of isolates was determined by agar dilution methods. Production of metallo-ß-lactamase (MBL) in Carb resistant strains was detected by enzyme activity or sodium mercaptacetic acid (SMA; Eiken, Tokyo, Japan) disk method. Presence of cfiA gene and IS were detected by PCR.
Results: In ca.1200 strains isolated from 1987 to 1994, we detected 38 resistant strains (B.fragilis 23, non-B.fragilis 15). MBL production was detected in only 9 strains of B.fragilis. MICs of imipenem in MBL (+) strains were higher (≥ 64 µg/mL) than that of MBL (-) resistant strains. MBL (+) strains were resistant to other ß-lactams including cephamaycins and ß-lactamase inhibitor(BLI)/ß-lactam, reflecting substrate specificity of MBL. Most of MBL (-) strains (especially in non-B.fragilis) were rather resistant to other ß-lactams including BLI/ ß-lactam and cephamycins. Resistant rate to clindamycin in those strains were ca 80%, although the rate in whole B.fragiis group isolated in the same period was ca.30%. Among 120 strains isolated after 2000, three MBL (+) B.fragilis and 7 MBL (-) carbapenem less-susceptible strains (MICs of carbapenems; 4~16 µg/mL) were detected.
Conclusions: 1) MBL (+) strains showed higher Carb MIC than that of MBL (-) Carb resistant. 2) MBL (-) type may isolate more often and distribute more widely in B.fragilis group than MBL (+) type. 3) MBL (-) type showed resistant to wide-range of antimicrobial agents. 4) Not only MBL (+) type but also MBL (-) type strains should be monitored.

Xylitol Resistance of Streptococcus mutans Appears To Be An Unexpected Benefit to The Human or Rodent Host

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Background: S. mutans is the prime cariogenic bacterium. Xylitol, a sugar substitute, inhibits its glycolytic metabolism, transmission from mother to child, and induction/severity of caries. However, xylitol-resistant mutants arise in the mouths of habitual consumers. There are no genotypic data on these mutants and no reports on their cariogenicity (virulence). S. mutans mutants have been constructed that are defective in fructose transport and one of these is also defective in xylitol transport. The mutant of one fructose PTS system that can transport xylitol (fruI$^-$) is notably resistant to growth and glycolytic inhibition by xylitol, unlike either its wild type (WT) progenitor, other fructose transport mutants, or reference mutans streptococcal strains.
Methods: We characterized the virulences of WT and engineered fructose transport-defective strains, one of which also fails to transport xylitol, using rats initially free of indigenous mutans streptococci. The rats ate a high sucrose diet 2000 supporting maximal cariogenicity. S. mutans UA159, its isogenic double crossover deletion mutants of fructose PTS (fruI$^-$), (fruCD$^-$), a double fructose PTS mutant (fruI$^-$/fruCD$^-$), and a sucrose phosphorylase mutant (gtfA$^-$) were simultaneously studied in TAN:SPFOM(OMASF)BR rats. Some rats were un-inoculated and some were inoculated with reference S. mutans strain 10449S.
Results: In two separate experiments, all S. mutans strains heavily colonized the rats, however, the recoveries of the fruI$^-$ mutant from sonicates of the rats' teeth post mortem were decreased, by comparison with other mutants and its WT. Mutants defective in fruI$^-$, fruCD$^-$, and fruI$^-$/fruCD$^-$ partially lost cariogenicity on enamel. But the fruI$^-$ mutant especially lost ability to induce decay deep into the teeth. The control gtfA$^-$ mutant did not lose virulence.
Conclusions: Fructose transport via the PEP-dependent fructose PTS of S. mutans UA159 contributes to virulence in sucrose-fed rats, but there are site-specific mutation effects on the ability of this cell to colonize the teeth. An engineered fructose PTS mutant (fruI$^-$) that fails to transport xylitol, is resistant to growth and glycolysis inhibition by xylitol, and loses some of its ability to colonize the teeth and to cause deep lesions. These results have been reported in extenso by Tanzer et al, J Dent Res 85,369-73,2006. The results suggest that emergence of xylitol resistance among S. mutans strains colonizing habitual xylitol users may, in fact, be of benefit to the host. Supported by grants UCHC 4-04020 and NIH DE-12236.

Fasudil: the New "Magic Bullet" to Thwart Malaria Mortality?

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Malaria, with more than 2 million deaths every year, remains a major world public health problem in terms of mortality. The onset of acute clinical manifestations of severe malaria, such as cerebral malaria (CM), occur rapidly and unpredictably, and may lead to coma and death within hours if left untreated. So far, severe malaria emergency treatments have mainly consisted of intravenous administration of antiplasmodial drugs, such as quinine or artemisinin. However, despite the high efficiency of those molecules in clearing the blood of parasites, 15-20% of lethality is still observed and a non-negligible proportion of survivors have malaria sequelae. Thus, current treatments that mainly rely on a 'direct antiparasitic strategy', are not sufficient and adjunctive anti-disease approaches are urgently needed.
Life-threatening malaria is overwhelmingly a result of the protozoan parasite Plasmodium falciparum, which infects red blood cells and confers them the unique ability to adhere massively on endothelial cells. There is increasing evidence that endothelium plays a key role in the pathogenesis of those malaria cases. Amongst these data is the demonstration that adhesion of Plasmodium falciparum parasitized red blood cells triggers inflammatory, oxidative stress and apoptotic cascades within endothelial cells of vital organs (e.g. brain, lungs, kidneys). We have exploited co-culture models of infected erythrocyte/endothelium interactions. We demonstrated that P. falciparum adhesion activates the Rho-kinase signaling pathway, a response highly involved in various human vascular diseases. A Rho-kinase inhibitor, Fasudil (HA-1077), a drug already in clinical use for the treatment of human cerebral ischemic stroke, was tested for its capacity to ameliorate endothelial functions. When added concomitantly with parasites, Fasudil was shown to abolish both NF-kappaB activation and endothelial apoptosis. More importantly, we showed that Fasudil could restored endothelial barrier integrity after endothelial cells were exposed to to P. falciparum adhesion.
This indicates that Fasudil may target efficiently endothelial pathogenic mechanisms even after severe malaria onset. Therefore Fasudil and and Rho-kinase inhibition-based strategies opens hopeful therapeutic venues in fatal malaria management.

The clinical role of the statins in surgical neurosciences

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Background: Statins are structural analogs of hydroxy-methylglutaryl Co A reductase, a restrictive enzyme in the cholesterol pathway.They are used for treatment of dyslipidemias and cardiovascular prevention. Animal studies show broad evidence of a neuroprotector effect. In humans is growing evidence of profit after subarachnoidal hemorrhage. After intracerebral hemorrhage (ICH) and traumatic brain injury (TBI) in humans, the evidence is scarce of yet.
Methods: We conducted a blind randomized clinical trial of patients with TBI, Glasgow 9–13, and intracranial lesions by CT scan. Patients with previous disability, hepatopathy or myopathy, multisystemic trauma, prior treatment in other clinic, surgical lesion or in brainstem were excluded. Each patient was allocated to rosuvastatin (RVS) or placebo 10 days. Main outcome, amnesia and disorientation time were evaluated. In another trial a prospective series of patients with ICH was treated with RVS and compared with a retrospective control group (relation 1:3). Exclusion criteria were history of neoplasm or TBI 4 weeks previous, non-hypertensive causes of haemorrhage, brainstem hemorrhage, steroid use, cranial surgery, initial hydrocephalus, and NIHSS (National Institut Health Stroke Skale) >30. Mortality and functionality at the time of discharge from the hospital were evaluated.
Results: The TBI study included 8 patients with RVS and 13 controls with similar basal characteristics. The use of RVS showed a reduction of amnesia time with an hazard ration of 53.76 (95% confidence intervals [CI], 1.58–1824.64). The ICH trial analyzed 18 patients with RVS and 57 controls with similar basal characteristics. The mortality rate in hospital was 1 (5.6%) with RVS and 9 (15.8%) in the control group. The odds ratios for a NIHSS >15 at discharge was 0.04 (95% CI 0.003-0.93).
Conclusions: Statins may reduce amnesia time after TBI and improve outcome after ICH. Immunomodulation seems to be involved. Further trials are needed in order to confirm this positive relation.

Bait bullets controlling Bacillus anthracis: from spore inhibition to toxins neutralization and prevention of spore- and/or toxin-induced cell death

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The potential use of glycoconjugates (carbohydrate-bearing polymers) is receiving considerable attention for biomedical and pharmaceutical applications. Among cellular targets by glycoconjugates, macrophages are ideal, since they play a central role in inflammation and act as reservoirs for microorganisms that are involved with deadly infectious diseases. In this context, we studied effects of glycoconjugates contributing to the inhibition of Bacillus spores and neutralization of toxins during phagocytosis. The effects of glycoconjugates were studied under the following three conditions, namely a) prior to, b) during, and c) following macrophage contact with B. anthracis recombinant toxins and spores during phagocytosis. Post-phagocytosis studies involved colony forming units, microscopic observation, macrophage viability, cytotoxicity, and Caspases release assays.
Glycoconjugates promote inhibition of spores and neutralization of toxins by blocking spore– and/or toxins–induced macrophage cell death, while increasing their activation level. This results in higher phagocytosis rate of spores, toxins neutralization, and macrophage viability. Even after being bound to spores/ toxins on one side, glycoconjugates serve as chemoattractants for macrophages on the other side. Macrophages become more prone to adhere to glycoconjugate-coated spores/toxins, resulting in decreased lactate dehydrogenase and Caspases, in increased nitric oxide production, phagocytosis, and killing of spores and neutralizing toxins.
The technique presented in this study may be helpful in finding glycoconjugates with bactericidal /antimicrobial and antitoxic properties.
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Micronutrient Interactions in Health and Oxidative Stress Conditions

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Background: The nutrition research during last two decades has evolved the newer dimensions of protective role of micronutrients in diseases related to oxidative stress and their interactions with each other.
Methods: Linkages of micronutrients have been extensively studied by our group in foods as well as through *in vitro* systems and in animal and human models.
Results & Conclusions: Riboflavin enhanced the bioavailability and uptake of food zinc and copper and had beneficial effect on iron and zinc utilization in weanling mice. Supplementation of nicotinic acid enhanced bioavailability of iron as observed through in vitro, animal and human studies. Apparent absorption of zinc in healthy adults was inhibited by thiamine and ascorbic acid and copper absorption was inhibited by phosphorus, calcium and niacin. In the human ileostomy model, dietary riboflavin had significant positive association while intake of iron and manganese showed weak negative associations with beta-carotene absorption from vegetarian meals. Factors influencing dietary and erythrocyte membrane zinc status in apparently healthy Indians were found to be phytic acid and zinc. Further, in a prospective study on supplementation of greens in healthy adults (40) multiple regression analysis indicated significant association of percent change in plasma zinc with intakes of zinc, riboflavin, iron, ascorbic acid, beta-carotene and copper. In a study on antioxidant potential of commonly consumed fruits and vegetables (96), ascorbic acid levels were associated with inhibition of lipid peroxidation ($r = 0.32$, $p<0.05$) and ferrous ion chelating ability ($r = 0.43$, $p<0.01$). In a cataract study, plasma TBARS, a measure of oxidative stress, showed a negative and highly significant correlation with lens iron($r=-0.53$,$p<0.0001$) and positive association with lens opacity ($r=0.30$,$p<0.05$). Diabetic cataract patients exhibited inferior plasma levels of selenium, zinc, ceruloplasmin, superoxide dismutase and retinol as compared to cataract patients without diabetes. Further, multiple regression analysis indicated association of intakes of iron, beta-carotene, ascorbic acid, polyphenols and inositol hexa phosphate with plasma oxidative stress ($p<0.01$) and intakes of iron, ascorbic acid and inositol triphosphate with lens oxidative stress ($p<0.01$). Insulin secretagogue activity was found to be significantly associated with zinc levels and marginally associated with oxygen radical absorbance capacity, copper or manganese levels.

Structure and Antimalarial Activity of Immunomodulator P-MAPA

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Background: Malaria is one of the world's most common diseases, caused by a four different parasites: Plasmodium vivax, P. falciparum, P. malariae and P. ovale, which is transmitted to humans by a female mosquito's bites. It is a public health problem today in more than 90 countries, inhabited by a total of some 2,400 million people - 40% of the world's population. Worldwide prevalence of the disease is estimated to be in the order of 300-500 million clinical cases while mortality due to malaria is estimated to be up to 3 million deaths each year, majority young children in Africa.
The drug resistance has reduced the effectiveness of several commonly used antimalarials, such as chloroquine. Although the artemisinin, a relatively new drug derived from wormwood, has shown promise in treating drug-resistant strains, in some parts of the world, artemisinin drugs are used indiscriminately for self treatment of suspected uncomplicated malaria - so we can expect to see malaria forms resistant to artemisinin soon according to WHO.
Methods: This work has as aim the investigations on immunomodulator P-MAPA, aggregated polymer of protein magnesium ammonium phospholinoleate-palmitoleate anhydride, isolated from Aspergillus oryzae, and relates its antimalarial activity. The 3D structure of protein part of P-MAPA and its antimalarial activity, as well as, the activities of P-MAPA´s micro- and nano- crystals in vitro, on Plasmodium falciparum, and in vivo in experimental infection models are our goals. Firstly, we are going to isolate, purify and characterize the protein part of P-MAPA that was shown to be small (16 kDa), arginine rich (35.2 %) and contain tryptophan (1.3 %). Therefore, its primary structure is going to be determined applying mass spectrometry, its secondary structure applying circular dichroism, while it is suggested that 3D structure elucidation can be achieved by fluorescence and nuclear magnetic resonance (NMR) techniques.
Results: At the moment, the P-MAPA crystals have been tested and the strong bioactivity against malaria was observed in vitro. A part of our research is dedicated to new P-MAPA formulations and we have obtained the nano-crystals and are optimizing the two nanonization method's conditions with aim to achieve as uniform as possible nanocrystals.
Conclusions: Hopefully, the P-MAPA could be presented as a new antimalarial agent.

CYP1A1, GST Gene Polymorphisms and Risk of Chronic Myeloid Leukaemia

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Background: Associations between polymorphisms for genes encoding enzymes involved in biotransformation of xenobiotics and susceptibility to several cancers have been shown in several studies. The aim of the present study is to investigate the influence of cytochromes P450 (CYP450) 1A1*2C and Glutathione S-transferases (GSTs) (T1 and M1) gene polymorphisms in susceptibility to chronic myeloid leukaemia (CML).
Methods: The frequency of CYP1A1 Ile/Val alleles and of GSTT1 and GSTM1 homozygous deletions was examined in 107 patients with CML and 132 healthy controls by PCR and/or PCRRFLP methods using blood samples.
Results: The frequency of CYP1A1 Val allele was found to be 19.2% in CML patients and 4.4% for controls, indicating that persons carrying this allele had an increased risk of CML (OR = 5.10, 95% CI: 2.60–9.97)(Table 1). The frequency of individuals carrying the GSTT1 null genotype was higher among CML patients (40.2%) compared to controls (19.2%) (OR = 2.82, 95% CI: 1.58–5.05; $p <0.001$). Therefore, GSTT1 present genotype may be a protective factor for CML. Although GSTM1 null genotype frequency was slightly higher in the patient group (44.9%) than in the controls (42.3%), this difference was not statistically significant (OR = 1.11, 95% CI: 0.66–1.86; $p = 0.693$). Individuals with GSTM1 null genotypes without the T allele have a 5.981 higher risk for CML than those who have the T allele (Table 2).
Conclusions: This data suggests that polymorphic CYP1A1 and GSTT1 genes appear to affect susceptibility to CML.

Mode of Action of a Naphthoquinone on Tuberculosis

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Background: Euclea natalensis is a shrub or small to medium size tree, which occurs in southern Africa. The roots of it are used by the indigenous people of southern Africa for various bacterial infections. It was showed that six naphthoquinones from this tree have activity against Mycobacterium tuberculosis. One of them, 7-methyljuglone, has very similar structure to menaquione. In the electron transport process of Mycobacterium species, electrons are transported by menaquinone. Aim: To generate homology model of one of the electron transport system enzymes, cytochrome b from M. tuberculosis, and to investigate the enzyme's possible interactions with the bioactive anti-tuberculosis compound isolated from plant Euclea natalensis.
Methods: Preliminary homology model of M. tuberculosis cytochrome b is calculated by the program MODELLER by using the crystal structure of R. sphaeroides.
Results: Preliminary results revealed that part of the ligand stigmatellin located in the cytochrome b of R. sphaeroides crystal structure has a very similar structure to our compound. In laboratory side, it was shown that the minimum inhibitory concentration (MIC) value on the H37Rv M. tuberculosis strain, of the compound is 0.5 □g/ml which is comparable to positive drug control rifampicin (0.125□g/ml) and better than ethambutol (1.250 □g/ml).

And what if we used for therapeutic drug delivery, the "magic bullets" that Dictyostelium discoideum cells expel as a multidrug resistance mechanism?

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In 1935, Raper brought the non-pathogenic eukaryotic amoebae Dictyostelium discoideum to the attention of the microbiological scientific community. More than 6 decades later, in 1999, the NIH (National Institutes of Health, USA) decided to add this micro-organism as a new model for biomedical research.
Already in 1990, we used Dictyostelium to find out by which mechanism the cells get rid of benzo(a)pyrene, the potent carcinogenic compound of tobacco smoke. We found that, like many human multidrug-resistant cells, Dictyostelium cells do have a multidrug resistance pump, the P-glycoprotein (170 kDa). However, this pump proved to be non-active and therefore not responsible for drug resistance in Dictyostelium cells. Thus, we had to search for a new mechanism mediating drug resistance. We found that Hoechst 33342 (HO342), a DNA-specific marker, was not vitally staining the nuclei, thus giving evidence of Dictyostelium cell nuclear resistance. We observed that when Dictyostelium cells are grown in the presence of HO342, they expel extracellularly vesicles loaded with the dye. Such a vesicular pathway of detoxification was indeed the new resistance mechanism we had been looking for.
The next question became: could these biological dye-loaded vesicles be used as a dye-vector to by-pass Dictyostelium nuclear resistance? Experimental data obtained with living naïve Dictyostelium cells (never in contact with the dye before) and also with K562r cells, a human leukaemia multidrug-resistant cell line, allowed us to propose Dictyostelium vesicles as a new promising non-viral drug delivery tool*.
As a first approach to therapeutic application, we chose hypericin, a photosensitizer intended for antitumoral photodynamic therapy. The biological loading of vesicles with hypericin was first controlled. Then, the vesicle-mediated drug transfer was studied, using two human cell lines as target cells, skin fibroblast (HS68) and cervix carcinoma cells (HeLa).
Altogether, these data probe that the Dictyostelium extracellular vesicles could be used as "magic bullets" for transferring a therapeutic molecule within human cells.

* Pending European Patent (DRITT SAIC Université Pierre et Marie Curie) no. WO2005004925 20-01-2005, I. Tatischeff, A. Alfsen, F. Lavialle, Extracellular vesicles from non-pathogenic amoeba useful as vehicle for transferring a molecule of interest to an eukaryotic cell.

Recombinant Expression of the Biosynthetic Enzyme for the Biotechnological Production of Tetrahydrocannabinol, a Revisited Magic Bullet from Marijuana

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Background: In recent years, tetrahydrocannabinol (THC), the psychoactive principle of marijuana, has attracted a renewed interest as a kind of Magic Bullet. Actually, in some countries, THC has been approved as a medicine for suppressing nausea and vomiting caused by cancer chemotherapy, and in addition, Sativex, a marijuana-based preparation containing THC, was licensed in Canada as a neuropathic pain reliever for adult patients with multiple sclerosis. In marijuana plants, THC generates from the acidic precursor tetrahydrocannabinolic-acid (THCA) via non-enzymatic decarboxylation. The biosynthetic enzyme, THCA synthase, is an attractive target for the biotechnological production of THC because THCA is readily decarboxylized into THC by heating. Aims: 1) To develop an effective expression system for THCA synthase. 2) To develop novel production system for THC using the recombinant enzyme.
Methods: The gene encoding THCA synthase was cloned into plasmid pPICZ for yeast transformation. The transgenic yeast *Pichia pastoris* was developed by homologous recombination with the vector at the alcohol oxidase promoter region. Cannabigerolic-acid (CBGA), the substrate for THCA synthase, was chemically synthesized, and fed to the *Pichia* culture for the enzymatic synthesis of THCA. The THCA produced was extracted with ethyl acetate, and decarboxylated into THC by heating at 120 degree C.
Results: THCA synthase was functionally expressed and secreted from *Pichia* cells. The optimal expression level (1.32 nkat/liter) was obtained when the cells were cultured in buffered complex medium containing supplements including riboflavin and casamino acids. When the substrate CBGA was directly added to the *Pichia* culture, THCA production could be detected, but in poor level (<10 % conversion). In contrast, the culture supernatant, from which the cells were removed, could effectively convert CBGA into THCA with a maximum conversion rate of ~98%, and a yield of ~33 mg/liter. THC was prepared by heat treatment of THCA extracted with ethyl acetate from the culture supernatant. The yield of THC was ~24 mg from 1 liter incubation.
Conclusions: 1) The culture supernatant containing the recombinant THCA synthase could synthesize THCA, which is decarboxylized into THC. 2) This is the first biotechnological production of THC.

Magic Bullets Made Easily Using Nano-particulate Membrane Bioreactors

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Background: Without genetic modification most secondary metabolites produced by fungi, actinomycets and members of the genus Bacillus are only produced when grown in solid-state culture. Aims: 1) To compare primary and secondary metabolisms in a membrane-surface liquid culture bioreactor called a nano-particulate membrane bioreactor (NMB) to sparged liquid cultures. 2) To assay the influence of membrane thickness on metabolic activities. 3) To scale the NMB up to a size suitable for commercial applications.
Methods: A variety of experiments were conducted to measure antibiotic biosynthesis, nutrient consumption and biomass growth in various NMB rigs, and were compared directly to sparges submerged cultures fed identical nutrient broths and incubated at the same temperature. The effect of varying the thickenss of the membranes in the NMB was also assayed with respect to primary and secondary metabolisms. The NMB was modified from a pouch form to a gill form, scaled up and applied to a variety of application.
Results: Penicillin yields, nutrient consumption and growth rates were significantly higher/faster in the NMB. Yields and production rates in the NMB were up to 2.4 and 6.0 times the greater than in sparged liquid cultures, respectively. The biomass was reused nine times in an NMB with the yields increasing through the experiment. After the first batch there was no lag before biosynthesis resumed, so primary and secondary metabolisms were concurrent. Scale-up has reached 1,150 m^2, capable of treating 3-40 kL/day depending on the application. The NMB can be repeatedly steam sterilized and can be made of materials compatible with pharmaceuticals manufacture.
Conclusions: 1) The concurrent solid and liquid phases in the NMB enables obligate aerobic microbes to grow in direct contact with air, while communicating with a liquid phase for easy control of pH, temperature and nutrient and precurssor concentrations, easy separation of products downstream, efficient biomass retention, and greater yields and production rates, which makes the NMB an ideal technology for biosynthesis of primary and secondary metabolites. 2) Productivity in the NMB increases with thinner membranes. 3) The NMB has been scaled up to be suitable for a wide variety of commercial applications.

Comparison of therapeutic effects of Reboxetine and Methylphenidate in children and adolescents with Attention-Deficit/Hyperactivity Disorder

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Background: Dysregulation of biogenic amines especially of norepinephrine and dopamine has been proposed in pathophysiology of attention-deficit/hyperactivity disorder (ADHD). According to this hypothesis patients with ADHD may benefit from drugs which increase noradrenalin such as reboxetine (a specific noradrenalin reuptake inhibitor).the main aim of this open-label study was to assess the efficacy and tolerability of reboxetine compared to methylphenidate in children and adolescents with ADHD.
Method: twenty boys diagnosed with ADHD as the reboxetine group and eighteen as the methylphenidate group, matched on their age, were enrolled in a 6-week open trial treatment. Assessment included the Conners' Parent Rating Scale-Revised, Short Version (CPRS-R-S) which was administered at baseline and weeks 2, 4, and 6. The dose of reboxetine was between 3 and 6 mg/day and of methylphenidate was 1mg/kg/day.
Results: it was found a significant reduction in ADHD symptoms as measured by CPRS-R-S in both groups ($P<0.01$), without any significant difference between two groups after 6 weeks of treatment. Reboxetine was relatively well tolerated. The most common adverse effects of reboxetine were decreased appetite, constipation, sleep problems, and dry mouth.
Conclusion: this open-label comparison trial suggests the efficacy of reboxetine in the treatment of ADHD in youth and it is comparable to methylphenidate. Controlled studies with larger samples are needed to confirm this finding.

Isatin (2.3-dioxo-indole) and its Analogues as New Antipyretic Agents

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Background: Most of the antipyretic drugs available are acting mainly on blocking the arachidonic cascade forming PGE2. From our previous experience isatin (2,3 dioxo-indole) was able to prevent hyperthermia by blocking the action of PGE2 also.
Methods: In the present work the action of isatin and isatin analogues (5-methylisatin, 6-hydroxyisatin, 7-ethylisatin, N-acetylisatin) have been tested on prostaglandin E2 (PGE2)-induced hyperthermia in mice and rats. Two forms of induced hyperthermia have been tested. When the PGE2 was given simultaneously with the isatin or analogues, the development of hyperthermia was tested, when the test compounds were given 3o min following PGE2 administration, the action on already existing hyperthermia was measured in mice and in rats. The temperature of the animals was measured in the colon.
Results: The results demonstrate that isatin in a dose of 12.5 mg/kg ip is able to block the initiation and and in a dose of 25.0 mg/kg ip attenuate the PGE2-induced existing hyperthermia in rats. In mice 3.12 mg/kg ip isatin can block the development of PGE2-induced hyperthermia, while in a dose of 12.5 mg/kg ip can attenuate the existing hyperthermia. 5-Methylisatin in a dose of 3.36 mg/kg ip can block the development of hyperthermia and in the dose of 13.44 mg/kg ip can attenuate the existing PGE2-induced hyperthermia in rats. In a dose of 0.21 mg/kg ip can block the initiation of hyperthermia and in a dose of 6.72 mg/kg ip attenuates the existing hyperthermia in mice.6-Hydroxy-isatin in a dose of lo.40 mg/kg ip blocks the development and also the existing hyperthermia in rats. In mice 5.2 mg/kg is able to block the development of hyperthermia and in a dose of 10.4 mg/kg attenuates the existing hyperthermia. 7-Ethylisatin in the dose of 0,112 mg/kg blocks the initiation and also the existing hyperthermia in rats. In mice both the initiation and also the existing hyperthermia can be blocked by the dose of 0,0288 mg/kg. N-Acethylisatin dose of O,O96 mg/kg blocks the initiation and the dose of 0,384 blocks the existing hyperthermia in rats. O,005 mg/kg blocks the initiation of hyperthermia, while the dose of 1.024 mg/kg blocks the existing hyperthermia in mice.
Conclusion. The results demonstrate that not only isatin but the isatin analogues can also block the PGE2-induced hyperthermia and that 7-ethyl- and N-acetylisatin are the most effective compounds in both blocking the development of hyperthermia and also in attenuating the PGE2-induced hyperthermia.

Activated iron(IV)-oxo structures in intermediates of heme enzymes and their models

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Background: Postulated high valent heme active sites of the cytochrome P450 enzymes during catalysis have not been readily observable. High reactivity and instability appear to make their physical characterization difficult. In contrast, high valent intermediates of the related heme enzymes known as peroxidase and catalase, have been under study for many years, and have been used to infer the nature P450 intermediates.
Methods: The activated ferryl forms of peroxidases from sources such as horseradish, turnip, baker's yeast, and other plant and mammalian sources were probed by resonance Raman spectroscopy. The two primary intermediates are known as compounds I and II, formed by two and one-electron oxidation of the ferric resting enzyme. Oxygen-18 and deuterium allow identification of Fe(IV)=O vibrations. In addition to the classical peroxidases, myoglobin has a low level of peroxidase activity. Myoglobin was studied as well since there is much interest in the characterization of ferryl myoglobin.
Results: Fe(IV)=O frequencies found for native peroxidases are lower than those found for synthetic model ferryl hemes. For peroxidases which retain activity over a wide pH range, such as horseradish peroxidase, the observed Fe(IV)=O frequencies are highly pH sensitive, indicative of ionization of an amino acid in the proximity of the heme active site. The pH dependent frequencies are also markedly sensitive to deuterium substitution.
Conclusions: It was not expected that Fe(IV)=O frequencies in proteins would be generally lower than those observed for the synthetic ferryl porphyrin models of compounds I and II. It has been recently speculated that the Fe(IV)=O groups in the protein active sites might be better described as Fe(IV)-OH. In actual fact, many of the observed Fe(IV)=O frequencies in the proteins are remarkably sensitive to deuterium substitution. However, it appears that the deuteration sensitivity is a result of hydrogen-bonding within the protein active site, that is not mimicked in nonaqueous synthetic systems. Hydrogen-bonding imparts partial single bond character to the protein Fe(IV)=O groups. But with only one known exception, the iron-oxo groups in proteins appear to still be best described as double bonds.

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Application of Population Pharmacokinetic Analysis and Monte Carlo Simulations Method in Drug Phenotyping. Assessment of Cytochrome P450 1A2 Activity in a Population of Adult Non-Related Caucasians from Sparse Data

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Background: Already published data of the authors have unambiguously demonstrated the ability of the Nonparametric Expectation Maximisation (NPEM) method of population pharmacokinetic modelling to deal with sparse data in estimating systemic caffeine clearance (CLS1) for monitoring and evaluation of cytochrome P450 (CYP) 1A2 activity in a population of adult non-related Caucasians. The results of this investigation have shown that NPEM analysis is a reliable and sufficiently sensitive method for clinical testing of liver function even in presence of reduced renal function, using CLS1 as a biomarker. On the other hand, these results have revealled the abilities of the NPEM method as suitable and relevant for large-scale epidemiological studies with regard to phenotyping cancer susceptibility of high risk populations by monitoring their CYP1A2 activity based on random sparse data. The method can deal even with mixed populations (smokers and non-smokers) in discerning multicomponent distributions with departure from normality, which the parametric methods have failed to do.
Methods: Large-scale epidemiological study was simulated using the method of Monte Carlo simulations (MCS) in order to explore the applicability of nonparametric population pharmacokinetic modeling in phenotyping of CYP1A2 activity by using of CLS1 as a biomarker. Simulated populations from 500 to 1000 subjects were studied. 250 simulated subjects were randomly sampled according to a 10 points sampling scheme and their simulated "measured" plasma caffeine concentrations were further submitted to nonparametric population pharmacokinetic analysis.
Results: The resulted distribution of CLS1 was clearly trimodal. Each of the clusters ("High", "Intermediate", and "Low") had normal distribution of CYP1A2 activity. Obviously, in no clinical setting it would be possible to sample randomly 250 subjects.
Conclusions: These results reveal the exceptional usefulness of MCS in drug phenotyping when dealing with clinical setting data which are, by their very nature, limited and sparsely distributed.

Efficacy of 1% topical cyclosporine in the treatment of severe vernal keratoconjunctivitis in childhood

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Introduction: Corticosteroids and high-concentrated cyclosporine eyedrops have been used for treatment of severe vernal keratoconjunctivitis (VKC) cases. The purpose of our study was to verify the efficacy of 1% topical cyclosporine in improving severe form of VKC in childhood and investigate for factors affecting the response to therapy.
Material and Methods: We conducted an open trial involving 197 children with severe VKC, who received topical cyclosporine 1% for 4 months. Ocular subjective symptoms and objective signs were scored in all children at entry, 2 weeks and 4 months. Skin prick tests and microscope endothelial cells evaluation were also performed; serum IgE and cyclosporine levels were assessed.
Results: The mean score values for severity of subjective symptoms and objective signs were significantly decreased after 2 weeks, and 4 months, compared with those at entry ($p<0.001$) in all children. Cyclosporine serum levels were not detectable at the end of therapy, nor were endothelial corneal cells damaged. Patients who started the therapy at the beginning of the disease and/or received long-term regimen of treatment with cyclosporine had a faster improvement of ocular signs and symptoms, compared to all other patients.
Conclusion: Our findings suggest that 1% cyclosporine concentration administrated topically at the beginning of the disease and for a long-term period might be the most effective treatment to control symptoms and local inflammation in severe forms of VKC in childhood.

Evolution of treatment of kala-azar during last 4 decades in Indai

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Abstract: In 1977 there was a massive epidemic of kala-azar with 100,000 patients and 30% of patients were unresponsive to the traditional regimen of sodium antimony gluconate i.e. 6 ml intramasuclar daily for 6 -10 days, starting with ½ ml per day and increasing ½ ml daily to reach 6 ml . By increasing the dose to 6 ml/im daily to 20 days without incremental dose led to cure rate of 92.6 %. This regimen was also approved by World Health Organization. But after some time even with this regimen cure rate decreased and we further rationalized the dose on body wt. basis and 20 mg/kg body wt. for 40 days of treatment gave the best results but toxicity of the drug also increased. 28 days regimen with this dose was agreed upon by World Health Organization and was also adopted by World Health Organization. Later on we found the drug very toxic and ineffications and advised for withdrawl of this drug. We worked also on pentamidine which was found less effective and very toxic causing permament dibetes and we recommended for its withdrawl from the market. We further worked on amphotericin B deoxycholate and found it very useful and recommended its use for general patients. In our experience 1 mg/kg body wt. of this drug given for 20 days gave the best results. We also worked how the toxicity of this drug could be minimized. We also worked on milltefosin and paromomycin Miltefosin has come in the market and paromomycin is underging phase IV trial. Our centre was involved in the trial of amphotericin B lipid complex (Ambisome) and found that even sigle dose of ambisome 15 mg/kg body wt. was well tolerated and cured all patients. We also worked on other oral drugs which were found effective by others but not found effective in our expericne and those drugs were discarded. We are also now working on a drug derived from the wild plant which has been found effective. An oral drug which could be found more effective and less toxic than the current drug in use needs to be developed and is the need of the hour. The principle of treatment and criteria for cure of PKDL, a major source of parasites were changed both with SAG and amphotericine B; 3 to 5 courses of SAG and amphotericine B eleared all lesions. No recurrence was encountered and no patient relapsed contrary to the previous belief. With 20- day regimen of amphotericin B for kala-azar treatment incidence of PKDL has also decreased. Thus there has been a complete change in the understanding of PKDL. Keeping in mind its prevalence in four states of India for the last hundred years. Kala-azar is a big challenge and we are attemting to eliminate it with the help of international co-operation.

Whooping cough vaccines: a public health and producer's perspective

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Background: Despite the high vaccination coverage in most of the Western world, the incidence of whooping cough has been increasing in all age groups for the last 2 decades. The global rise in whooping cough cases did not lessen after the introduction of acellular vaccines a decade ago, or the introduction of booster vaccinations in toddlers. Currently registered vaccines are not well equipped to overcome the rising incidence of whooping cough for a number of reasons. Therefore, there is a definite need for an improved whooping cough vaccine.
Methods: This paper selected the following characteristics of an improved vaccine. An improved whooping cough vaccine should (1) enable all age groups to be vaccinated with *B. pertussis* circulating strain antigens, (2) protect against whooping cough induced by *B. parapertussis*, (3) enable infants to be vaccinated earlier, (4) cause minimal adverse events after repeated vaccination, and (5) protect longer than currently registered vaccines. A number of scenario's and corresponding vaccine compositions that comply with (part of) these characteristics were examined, using time to market, costs and risks as constraints.
Results & conclusions: The most likely candidates to fulfill all characteristics are oral or intranasal vaccines consisting of inactivated whole *B. pertussis* cells, given the fact that an oral vaccine has already shown proof of protection in a phase III study without adverse events, and that an intra-nasal vaccine has shown proof of concept in a phase I study. Live attenuated vaccines fulfill many of the 5 characteristics as well, but will most likely take longer to reach the market. At this point it is not clear if a *B. parapertussis* component should also be included in an improved whooping cough vaccine, and what the cost-benefit ratio would be.

Nephrotoxicity of immunosuppressive drugs, new insight

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Background: Organ transplantation is the best treatment, if not the only, of end stage organ failure. However, even though the incidence of acute rejection episode has dramatically lowered after renal transplantation with a short term graft survival above 90 %, long-term results have not improved consequently. Conversly, non renal transplant, chronic renal failure (CRF) is a common complication. One of the major causes of CRF, namely nephrotoxicity, is a major side effect of the immunosuppressive drugs used and may precluded long-term outcome. This side effect is well-recognized for calcineurine inhibitors but sirolimus (SRL), a mTOR inhibitor, also exhibit nephrotoxicity especially after delayed graft function.
New diagnostic tools can be used. They include pharmacokinetic approach such as abbreviated aera under the curve, automatic quantification of interstitial fibrosis or pharmacometabonomic.
New insights of nephrotoxicity mechanisms also have been demonstrated. For calcineurine inhibitors, in vivo and in vitro data demonstrate that P-glycoproteine may play a critical role in protecting renal epithelial cells from cyclosporine A (CsA) toxicity. Regarding the molecular mechanisms, transcriptional profiles of human proximal tubular cells exposed to CsA found that it preferentially alters biological processes located at the cell membrane, such as ion transport or signal transduction. Wide expression analysis suggested that CsA may induce an endoplasmic reticulum (ER) stress in tubular cells in vitro and in vivo, because of cyclophilin A inhibition. Furthermore, CsA induces epithelial phenotypic changes reminiscent of an incomplete epithelial-to-mesenchymal transition in a TGF-β independent manner, as did various ER stress inducers. Finally, in primary cultured human tubular cells CsA induces autophagy dependant of ER stress. SRL modifies biological processes within the nucleus and related to transcriptional activity. It inhibits the proliferative response to mitogenic stimuli, and causes cell cycle arrest in the early G(1) phase, by a nonspecific process due to inhibition of the p70(S6k) pathway and by a direct effect on cyclin D3 mRNA stability.
Finally, new therapeutic approach such as calcineurine inhibitors mimization conversion or avoidance, have demonstrated contradictory results because of the increased risk of acute rejection.

Evaluating our Performance: Tissue Penetration Paradigm Shift

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The term paradigm shift, as introduced by Thomas Kuhn, describes a change in basic assumptions within a ruling theory of science. Such a change in a certain thought-pattern is usually the result of a long process. Applying the paradigm shift model to the measurement of concentrations of anti-infective drugs at the site of infection, we indeed observe a remarkably slow shift in thinking and acting even in the face of compelling reasons for change. For several decades anti-infective drug concentrations at the site of infection have been primarliy measured by taking a whole tissue biopsy, grinding it, determining the total concentration in the homogenate, comparing it with the corresponding blood sample, and then judging a drug's clinical value and performance from such a measurement. As has been shown, homogenizing biopsy samples results in a mixture of distinct pharmacological compartments, of bound and unbound drug, and, thus, fails to give meaningful information about concentrations at the infection site. Surprisingly, despite our knowledge of the many well-known problems with interpreting tissue concentrations from biopsy samples, this method remains in fairly common use over many years after the flaws were described. According to Kuhn's definition of a paradigm shift, the anti-infective research community is - like other research communities - reluctant to change and slow to adopt new thinking. This can be attributed to some extent to the lack of reliable alternatives or to challenging issues with new methods. However, if the new paradigm of repeatedly measuring free concentrations in distinct pharmacological compartments - such as extracellular fluid of tissues or various body fluids - is generally accepted, thoroughly explored and executed, the old paradigm will be replaced. We seem to be close to this paradigm shift and thus to more accurate measurement and interpretation of tissue concentrations.

Magnetic Resonance Imaging 'Pathfinder' Molecules For Use in Optimizing Antibody and Gene-Directed Enzyme-Prodrug Therapy (ADEPT/GDEPT) Treatments

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Background: A number of Antibody and Gene Directed Enzyme Prodrug Therapy (ADEPT/GDEPT) systems have been developed over the past two decades in order to target anti-cancer drugs to tumour cells and several of these have entered clinical trials. Here we report new magnetic resonance imaging (MRI) contrast agents that have been designed to function as 'pathfinder' molecules and allow the non-invasive visualization of the localization and catalytic activity of the enzyme carboxypeptidase G2 (CPG2) used in these prodrug activating systems.
Methods: Contrast agents are used in ~50% of all human MRI scans in order to improve the contrast between different tissue types in the images generated. Positive contrast agents cause a reduction in the T1 relaxation time of the hydrogens in water molecules that are coordinated to the metal in the contrast agent. We have modified the clinically used gadolinium(III) contrast agents Gd(III)-DOTA (Gadoterate meglumine:Dotarem®) and Gd(III)-HP-DO3A (Gadoteridol: ProHance®) to incorporate enzyme cleavable appendages that protect the remaining coordination sites of the gadolinium(III) ion from binding to water molecules until they are cleaved by the enzyme. We have measured the difference in relaxivity for the 'pro-contrast agent' and the hydrolysed product that is able to bind one or two water molecules and so 'lights-up' in the MR image
Results: We have identified gadolinium(III)-based contrast agents that exhibit a 3-5 fold signal enhancement after being cleaved by MFECP1, a recombinant antibody-enzyme fusion protein of an anti–carcinoembryonic antigen single-chain Fv antibody and the bacterial enzyme carboxypeptidase G2. The structure of these is being optimized to improve the compound's stability and rate of CPG2 hydrolysis
Conclusions: New enzyme-activated MRI contrast agents have been produced that once evaluated for use in humans could be utilised to optimize ADEPT or GDEPT treatments on a patient by patient basis.

Angiotensin Converting Enzyme Inhibitors Determination in Plasma by Enzyme Kinetic and High-Performance Liquid Chromatography

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Background: The analysis of Angiotensin Converting Enzyme (ACE) inhibitors in plasma is an essential part for ACE inhibitors pharmacokinetics and bioequivalence determination. A reliable and convenient analytical method is needed. Aims: 1) To develop the simple analytical method for analyzing ACE inhibitors in plasma having enalapril and captopril as the representative compounds, by enzyme kinetic principle and high-performance liquid chromatography (HPLC). 2) To apply the method for determining the concentration of enalaprilat in volunteers' plasma after oral administration of enalapril.
Methods: The concentration of ACE inhibitors in plasma was determined by analyzing hippuric acid by HPLC, using 2-methyl hippuric acid as the internal standard. Hippuric acid is the product from the reaction between exogenous ACE and hippuryl-histidyl-leucine. The HPLC analysis of hippuric acid was carried on a C-18 column, and quantified by UV detector at 228 nm. This analytical method was verified by perfoming a bio-analytical method validation. The method was then used for analyzing enalaprilat concentrations in twelve healthy male volunteers after oral administration of enalpril to determine pharmacokinetic parameters.
Results: The proposed method is simple, neither sample extraction nor derivatization is required. The HPLC analysis consumes less than 6.5 minutes per sample. By using human plasma as exogenous ACE for ACE inhibitors and analyzing hippuric acid, the concentration of plasma ACE inhibitors could be determined with the lowest concentrations of enalaprilat and captopril of 3.0 and 10.0 ng/ml, respectively. The relative standard intra-day and inter-day deviations of each compound in plasma were less than 9%. The percentage of bias was less than ± 1% which confirmed the accuracy of the method. The method has been proven for its reliability in analyzing plasma enalaprilat concentration after enalapril administration
Conclusions: 1) The concentration of ACE inhibitors in plasma could be simply determined by analyzing hippuric acid, the product of ACE and substrate reaction. 2) The use of human plasma as exogenous ACE for enzyme kinetic analysis of ACE inhibitors is convenient and accurate. 3) The method could be applied for all ACE inhibitors. 4) The method was successfully utilized in pharmacokinetic and bioequivalent studies of enalapril.

Antifungals in the Treatment of Candidiasis Eruption in Oral Autoimmune Diseases

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Background: Candida infection has been reported as the common lesion eruption during treatment of oral autoimmune diseases such as oral lichen planus (OLP), lupus erythematosus (LE), pemphigus vulgaris (PV) and mucous membrane pemphigoid (MMP) with topical and systemic steroids. Aims: 1) To study the incidence of candidiasis eruption after treatment with steroids. 2) To investigate the effectiveness of topical antifungals. 3) To know the side-effects of topical antifungals.
Methods: A retrospective study included 297 patients (female=236; male=61), age ranged between 18-80 years (Mean±SD=48.69±13.46). Data of the patients with OLP (268), LE (6) and MMP (6) cases randomly treated with topical steroids such as fluocinolone acetonide, clobetasol propionate, triamcinolone acetonide and dexamethasone were analyzed. In addition, 17 cases of PV including drugs-induced PV treated with topical and systemic steroids were included in this study. All cases were diagnosed clinically and confirmed by histopathological or immunofluorescent studies. The first candidiasis detection after treatment with steroids was recorded and investigated by KOH 10%, Periodic Acid Schiff stain (PAS) or Candida culture. Topical miconazole and nystatin were used in 40 and 25 cases with candidiasis respectively. Oral findings during treatment in all cases and side-effects were recorded. The data were processed by SPSS 13.0 for Windows.
Results: Pseudomembranous candidiasis was the most common lesion found on the areas of treatment in 73 out of 297(24.58%); comprising 66/268 (24.63%) in OLP, 5/17 (29.41%) in PV, 2/6 (33.33%) in LE patients but no candidiasis was found in all MMP patients. The duration of candidiasis eruption in those lesions after treatment with steroids varied from 0.23-87 months (Mean±SD=8.40±15.69). However, candidiasis could be eliminated by topical miconazole and nystatin in all cases without any side-effects in long-term follow-up.
Conclusions: Our study confirms that steroids can induce candidiasis after treatment of various oral autoimmune diseases and topical antifungals can be effectively treated candidiasis without any side-effects.

Innovative Method For Quality Control of High Molecular Weight Semi-synthetic Vaccines

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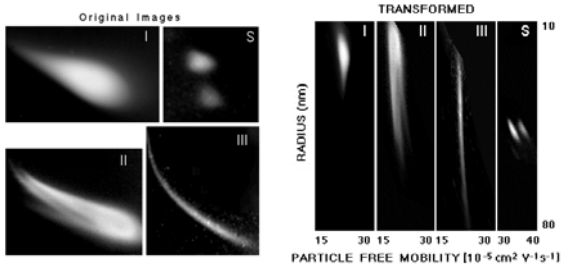
Background: Robbins, Schneerson et al. developed conjugated meningitis vaccines (Hib) for infants [1]. Earlier preparations varied in immunogenicity. For accelerated testing of vaccines, analytical methods based on physical parameters were developed from 1983 - 95. These techniques [2] remain a promising tool for vaccine quality control and for predicting vaccine effectiveness (recently reviewed in [3]).
Methods: Horizontal 2-D submarine-type agarose electrophoresis was employed, a method developed by Serwer [4] for the separation of intact viruses. Computer programs ElphoFit and GelFit were used for data analysis and image processing [2].
Results: Original images (left) show characteristic patterns for vaccine preparations (I-III) and two carboxylated polystyrene samples (S) used for standardization. These images were transformed (right) to a coordinate system of particle radius *vs.* free mobility (surface net charge). No distinct zone patterns can be seen (I-III), since sizes of vaccine particles vary continuously over a wide range (polydisperse) due to randomizing processes in the vaccine preparation (crosslinking and sonication).



Conclusion: 1) The vaccine patterns are a fingerprint of the preceding vaccine preparation process and, therefore, can be used for purposes of quality control. 2) Results are available within 1-2 days, whereas immunological testing may take several weeks. 3) Samples II and III with particle sizes larger than 30 nm radius were effective vaccines. Sample II contains a mixture of vaccine batches. 4) The technique cannot only be used for Hib conjugated meningitis vaccines, but also for other high molecular weight vaccines.

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The in vitro effects of Thymoquinone on human endometrial adenocarcinoma cells

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Background: In this study, it was investigated whether Thymoquinone (TQ) has the effect on women endometrial adenocarcinoma cells as in vitro.
Methods: It was used frozen vial human endometrial adenocarcinoma (KLE) cells that obtained from American Type Culture Collection (ATCC) in this study. KLE cells were treated with TQ for various dosages.
Results: According to experiments, it was founded that TQ has toxic effect in all dilutions until that 300 micro molar (µM). Especially, TQ had blocking effect on growing in number of KLE cells.
Conclusions: It was determined that TQ that composition of Nigella sativa has blocking effect on growing in number of endometrial adenocarcinoma cells as dose depending in cytotoxicity experiments that in vitro.

Dissecting the DNA Base Excision Repair Pathway: Implications for Cancer Therapy

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Background: The base excision repair (BER) pathway removes DNA bases damaged by oxidizing and alkylating agents, as well as mispaired bases. The first step of BER is initiated by DNA glycosylases that recognize and excise aberrant bases. Subsequently, the resulting abasic site is processed by other specialized BER enzymes that generate a single nucleotide gap and replace the missing nucleotide. The BER pathway is clinically important as it prevents deleterious mutations and also processes DNA lesions produced by anticancer drugs. Thymine DNA glycosylase (TDG) is a BER enzyme dedicated to the repair of damaged cytosine-guanine (CpG) dinucleotides. TDG and other BER enzymes have been implicated in epigenetic control of gene expression by gene-specific CpG demethylation. TDG also regulates gene expression by direct interaction with the transcription machinery. Interestingly, this versatile enzyme also mediates removal of 5-fluorouracil from DNA and may determine the sensitivity of cells to this widely used anticancer drug. TDG is posttranslationally modified by sumoylation, phosphorylation and acetylation. In exploring the potential of TDG as a drug target, we have sought to decipher how these modifications regulate various TDG functions.
Methods: We have employed cell imaging techniques, cell-based reporter gene assays and biochemical analysis to investigate the regulatory roles of TDG posttranslational modifications.
Results: We demonstrate that posttranslational modification of TDG dramatically affects TDG subnuclear localization as well as interactions with DNA and accessory proteins, thereby altering the transcriptional and DNA repair functions of this enzyme. Importantly, we have identified specific kinase signalling pathways responsible for phosphorylation of TDG in living cells and show that phosphorylation acts antagonistically to acetylation to regulate DNA damage processing. These findings have allowed us to design mutant versions of TDG that display more potent DNA repair functions.
Conclusions: These findings suggest that multiple post-translational modifications and therefore different signaling pathways regulate the biochemical properties and subcellular localization of TDG. The interplay of these covalent modifications allow for exquisite regulation of a DNA repair pathway integral for genome stability

The Acute Effects of Statins in an Experimental Model of Renal Ischemia-Reperfusion Injury

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Background: Renal ischemia-reperfusion (I/R) injury with a high mortality rate and unresolved questions in pharmacotherapy remains one of the leading causes of acute renal failure. We aimed to compare the acute effects of different statins in an established model of renal I/R injury.
Methods: Adult male Wistar rats, anesthetized with sodium thiopentone and subjected to renal I/R injury (45 min of I + 4 h of R), were pretreated with simvastatin or pravastatin (1 mg/kg, i.v.), 30 min before I, 30 min before R or 5 min before R. Control rats (subjected to I/R injury) and sham-operated rats were pretreated with appropriate solvent only (10% DMSO or saline). Blood and kidney tissue samples were taken at the end of experiment and selected parameters of glomerular and tubular function were assessed. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test.
Results: Simvastatin-treated rats had significantly reduced serum creatinine concentrations, fractional excretion of sodium and total histological score in comparison with the controls (up to 60%, 80% and 40%, respectively). The acute protective effects of simvastatin (1 mg/kg) did not depend on the time of injection and the dose used. On the other hand, pravastatin (1 mg/kg) was significantly more effective than simvastatin in reducing I/R-induced changes of glomerular and tubular function, especially regarding total histological score.
Conclusion: The acute pretreatment with a single dose of statin may ameliorate renal impairment and allow earlier recovery from I/R injury. However, it seems that statins are not equally effective in reducing such an injury.

Clinical Role of Fluorouracil Metabolizing Enzymes and Optimal Duration in Chemotherapy with Tegafur

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Objectives: Response rates of 5-fluorouracil (5FU) and tegafur likely differ individually due to differences in the enzyme activities for anabolism and catabolism. Orotate phosphribosyl transferase (OPRT) is an essential enzyme for activation of 5FU. Dihydropyrimidine dehydrogenase (DPD) is a rate-limiting enzyme for degradation of 5FU. To obtain a high percentage of response without side-effects, it would be important to evaluate the enzyme activities and to estimate the optimal duration of chemotherapy.
Patients and Methods: The study included 160 patients, whose colorectal cancer (Stage II to IV) were treated with uracil and tegafur (UFT) after surgery. (1) OPRT and DPD expressions were evaluated using immunohistochemistry. Relationships between their expressions and clinicopathological features. Survival curves were calculated using Kaplan-Meier method. (2) Using the collagen gel droplet embedded drug sensitivity test (CD-DST), we estimated the optimal duration of chemotherapy. Area under curve (AUC) was calculated with the inhibition rates assessed in variety of time and concentration in the tests. The AUC value for 50% inhibition (AUC_{IR50}) was also calucated. The AUC_{24hr} of 5FU infusion (250 mg/mm2/day), UFT (400 mg/m2/day) were about 1.7 and 1.4 (micro-gram x hr/mL). Based on these values, the duration to attain the AUC_{IR50} for 5FU and UFT were estimated.
Results: (1) OPRT expression showed a negative correlation with advances in cancer stage, though DPD expression showed positive correlations. The patients survival were better in those OPRT(+) than in those OPRT (-) (p=0.004). The patients survival were better in those DPD(-) than in those DPD(+) (p=0.008). In regard to the combination of these expression, the best survival curve was obtained for the OPRT(+) DPD(-) group and the worst was OPRT(-) DPD(+) group. (2) The AUC_{IR50} in 38% of the patients ranged **<** 100**;** 43% ranged 100-1000; 13% ranged 1000-10000; and 6% ranged > 10000 respectively. Therefore, the duration to attain the AUC_{IR50} by 5-FU infusion ranged < 10 weeks in 37% patients; 10-100 weeks in 44 %; and longer than 100 weeks in 19% respectively. The durations to attain the AUC_{IR50} by UFT ranged < 6 months in 55% patients; 6-12 months in 13 %; and longer than 12 months in 32% respectively.
Conclusions: OPRT expression related with better prognosis, although DPD expression were related with poor prognosis. The use of several determinants of response may identify a high percentage of responding patients. The optimal duration of chemotherapy with 5FU or tegafur may be estimated using CD-DST, though it differs in patients.

Developing a magic bullet against P-glycoprotein-mediated drug resistance by exploiting mechanism

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Background: Multidrug resistance (MDR) is a major obstacle for the treatment of cancer as well as bacterial infections. Efflux pumps are often the root cause of MDR. The prototypical human multidrug resistance efflux pump P-glycoprotein (Pgp) couples drug export to ATP binding and hydrolysis. Details regarding drug trajectory, the molecular basis for coupling, and factors governing transport rate/efficiency remain unknown. Our goal is to reveal these details. Rhodamine dyes have been used as as drug mimics to assay Pgp-mediated transport. Herein, we employ novel synthetic analogues of Tetramethylrosamine (TMR) to probe Pgp mechanism. A unique advantage is our ability to tease apart the capacity of a drug to confer coupling to ATP catalytic sites versus transport rate/efficiency.
Methods: A library of TMR analogues was constructed and their effect on purified lipid-activated mouse MDR3 Pgp ATPase was determined. Coupling to ATP catalytic sites was also determined by their ability to promote ADP-Vi trapping, ATP occlusion in "catalytic carboxylate" mutant Pgp, as well as through inhibition of verapamil-dependent ATPase. Effect on drug transport was measured *in vivo* with Madin-Darby Canine Kidney monolayer cells that express Pgp (MDCK-MDR1).
Results: Nearly three orders of magintude variation in ATPase stimulation within the library was apparent. Importantly, the concentration of TMR required for coupling to the ATP site does not clearly corelate with turnover rate. In some cases, the ability to promote ATP occlusion is associated with robust turnover. Several other analogues stimulate ATP occlusion relatively well but displayed very slow turnover rates. This class also appeared relatively effective as Pgp inhibitors/modulators *in vitro* and *in vivo*.
Conclusions: Drug recognition may not be apparent from ATPase stimulation which may manifest as equivalent to (or below) basal values. Promotion of ATP occlusion appears to reveal the true affinity of a drug for Pgp and this does not corelate with transport rate. Molecules that promote ATP occlusion effectively yet confer slow turnover rates are good leads for development of competitive inhibitors.

Bacterial resistance and influence on optimal choice of antibacterials

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Background: Several studies have reported high rate of antimicrobial resistance among isolates from hospital. The aim of this study was review the pathogens associated with nosocomial infections at Clinic for abdominal surgery, Clinic for urology surgery, Clinic for Orthopedic surgery and Clinic for Resuscitation and Anesthesia Clinical Center of Vojvodina.
Methods: This investigation includes all bacterial strains isolated from patient's material collected routinely from Clinic for abdominal surgery, Clinic for urology surgery, Clinic for Orthopedic surgery and Clinic for Resuscitation and Anesthesia Clinical Center of Vojvodina. The investigation was done in period of 01.01.2006. to 31.12.2006. year. The places from which isolates were collected are: surgery lesion, urine, patient's blood.
Results: The results of analysis have shown that the collected data are insufficient for conclusions. The percentage of positive results was too low to enable to make recommendations. The list of antibiotics used for determination of sensitivity should include more first choice antibiotics according to international recommendations. The most frequently bacteria were tested to second and third choice antibiotics, to which the resistance rate is concerning high.
Conclusions: There is evidence for huge number of fecal contamination bacterias isolated from lesion and other places. Because of that there is need for urgent antisepsis and disinfection measures that we can lower contamination and need for antibiotics.

Multiple Targets of Pyrvinium Pamoate in Mammalian and Parasite Mitochondria

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Background: Pyrvinium pamoate, an anthelmintic, interferes with absorption of glucose by the helminths. Recently, we have reported that pyrvinium pamoate inhibited cancer cell survival under glucose starved culture, shown as anti-cancer compound in mammalian. The differences in energy metabolisms between the mammalian and helminths have been thought to be a target of pyrvinium. One of the important metabolic systems in helminths, NADH-fumarate reductase system, which is the final step of the phosphoenolpyrvate carboxykinase (PEPCK)-succinate pathway, is plays and important role in the anaerobic energy metabolism. It has been thought that the effect of pyrvinium is from inhibition of the fumarate reductase activity, which appears to be the key enzyme in NADH-fumarate reductase system. This study examined which enzymes were related to these effects both of the mammalian and the parasite mitochondria.
Methods: Enzyme activities were measured in Ascaris sum, bovine heart and cancer cells' mitochondria cultured with tumor-mimic conditions.
Results: Pyrvinium pamoate inhibited not only fumarate reductase (Complex II) but also NADH-quinine reductase (Complex I) in the anaerobic respiratory chain. Moreover, as same as in parasite, pyrvinium pamoate inhibited Complex I activity in mammalian mitochondria. However, compared with A. suum, pyrvinium showed different effect on mammalian Complex II, especially in cancer cells cultured with tumor-mimic conditions.
Conclusions: The species-specific, tumor-specific inhibitory effects by pyrvinium were caused by the different effects on Complex II activity in the mammalian and parasite mitochondria. These observations with pyrvinium pamoate emphasize the difference in metabolic systems between the parasite and the hosts, and the tumor in mammalian.

Aminoglycoside Derivatives as Drug Transporters: Delivery Magic Bullets?

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Background: A major obstacle for the development of new therapeutic agents is drug delivery. Many drug candidates never make it to the clinic because of inappropriate pharmacokinetic characteristics and sluggish uptake. Delivery procedures based on passive diffusion across cell membranes encounter problems due to charged groups, and carriers that exploit endogenous membrane transporters limit the size of potential drug candidates. Low MW delivery vehicles that facilitate the uptake of diverse molecular cargos are highly desired. Guanidinoglycosides, a family of synthetic derivatives where all the ammonium groups of aminoglycosides have been converted into guanidinium groups, efficiently facilitate uptake of cargo into mammalian cells.
Methods: To quantitatively examine cellular uptake using flow cytometry, fluorescent conjugates of biotinylated guanidino-neomycin with streptavidinylated-phycoerythrin-cychrome were incubated with wild type and mutant CHO cells defective in glycosaminoglycan assembly. To examine the ability of guanidino-neomycin to deliver large proteins into cells and release them in active form, saporin was conjugated to guanidino-neomycin-biotin and cell killing was evaluated. This conjugated toxin was also used to examine the susceptibility of tumor cell lines to these carriers. Enzyme replacement therapy was explored in MPS fibroblasts with guanidinoneomycin conjugated to □-gluocoronidase.
Results: (1) the cellular binding and uptake of guanidinoglycosides at low concentration depends exclusively on heparan sulfate (in contrast, the uptake of arginine-rich peptides follows multiple pathways); (2) guanidinoglycosides are capable of cellular delivery of high MW and bioactive cargo such as enzymes and protein toxins; (3) effective guanidinoglycoside-mediated delivery can be achieved at low transporter concentrations, with little or no cellular toxicity; and, preliminarily, (4) certain tumor cell lines (particularly breast cancer) show high susceptibility to guanidinoglycoside-linked toxins.
Conclusions: Guanidinoglycosides are promising low MW cellular transporters with high selectivity towards heparan sulfate. These carriers can be used as a screening tool to explore the surface glycobiology of tumor cell lines as well as for the targeted delivery of bioactive molecules (e.g., enzymes).

Change in Immunisation Schedule and Sudden Infant Death Syndrome in Hungary

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Infant mortality in Hungary was higher than in other European countries; however, the reported incidence of Sudden Infant Death Syndrome (SIDS) has been lower than those for Western Europe and the United States. Childhood immunisation has been reported to be a protective factor for SIDS. In Britain, the change to an earlier immunisation schedule for diphtheria, pertussis, and tetanus appeared to be associated with a shift in the age distribution of SIDS. In 1999, immunisation for *Haemophilus influenzae* type b (Hib) was introduced for Hungarian infants at the age of 2 months. Data for total infant mortality and SIDS in Hungary was analysed between 1990-2002. Infection was the major cause of death among Hungarian infants followed by SIDS. Following introduction of Hib immunisation, there was a decrease in deaths due to meningitis from an average of 3.5% of all infant deaths between 1990-1998 to an average of 1% of all infant deaths between 1999-2002 (p=0.00). There was also a significant decrease in the proportion of SIDS in the age range ≥2 months from 48% in the earlier period to 39% after introduction of the vaccine (p=0.03). The decease in SIDS might be due to decrease in unrecognised Hib infections or to induction of antibodies by the tetanus toxoid to which the Hib polysaccharide is conjugated that are cross reactive with bacterial toxins implicated in SIDS.

Generating C-reactive protein inhibitors for the treatment of cardiovascular disease

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In recent years, the role of C-reactive protein (CRP) in cardiovascular disease has been a lively topic of debate. The reasons for this interest can be summarized as follows. First, CRP has been identified as a powerful cardiovascular risk marker. Second, CRP may not only be a cardiovascular risk marker but also a risk factor, i.e., it may be causally involved in atherogenesis and its sequelae. Third, if it is indeed a cardiovascular risk factor, CRP may be a therapeutic target for primary or secondary prevention of cardiovascular disease. The medical and economic impact of these possibilities is evident. However, despite considerable research, the key questions concerning the role of CRP in cardiovascular disease remain unanswered. In this presentation we summarize the evidence for an active role of CRP in atherosclerosis and provide an update on the development of CRP-inhibitors for the treatment of cardiovascular disease.

Use of Pharmacodynamic Modeling to Predict Efficacy of Doripenem, a Class 2 Carbapenem recently Licensed in Europe

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Background: Two class 2 carbapenems (meropenem and imipenem/cilastatin) and one class 1 carbapenem (ertapenem) have been licensed in Europe for a number of years. With the advent of the third class 2 carbapenem doripenem aspects of pharmacodynamics, dosage and clinical efficacy deserve consideration.
Methods: Experimental publications concerning the pharmacodynamics against relevant target pathogens under different dosing regimens are reviewed. Clinical results using these dosing regimens are presented.
Results: Experimental infection studies in small animals have shown that the time above MIC (t>MIC) is the relevant pharmacodynamic parameter predicting therapeutic response for ß lactam agents. Specifically, for carbapenems, it has been shown that t>MIC must reach a value of at least 30-40 % of the dosing interval. Interestingly, this value was not considerably different for application frequencies of 1 x , 2 x or 3 x daily. Relevant pharmacokinetic parameters in humans for a 500 mg i.v. dose of doripenem are: Cmax 46.9 ± 7.4 mg/L, tmax 0.5 hrs, AUC 0-∞ 59,3 ± 7.2 mg•h/L, Vss 11.0 ± 1.7 L. Following a 500 mg dose infused over 0.5 h, mean serum levels exceeded MIC values of 16 mg/L for 1 h (t>MIC 12.7%), 4 mg/L for 3 hrs (t>MIC 37%), and 1 mg/L for 5.9 hrs (t>MIC 73%). Prolonging the duration of infusion to 4 hrs resulted in a 99% probability to exceed a t>MIC value of 40 % for pathogens with a MIC of 4 mg/L. For pathogens with higher MICs (8 mg/L), doses of 1000 mg with infusion periods of 4 hrs must be used to achieve the same 99% probability of target attainment.
Conclusions: On the basis of pharmacodynamic modeling and given the fact that most enterobacteria exibit MICs ≤ 1 mg/L, doses of 500 mg with an infusion time of 1 h are recommended for indications where P. aeruginosa is not considered. In clinical studies, this dosage regimen resulted in therapeutic response rates of 81.3 % (nosocomial pneumonia), or 85.9% (intraabdominal infection) of patients, respectively. For pathogens with higher MICs such as P. aeruginosa (typical MIC range 2-16 mg/L), doses must be increased or infusion periods prolonged. In general, for effective therapy of this organism, doses of 1000 mg given every 8 hrs for an infusion period of 4 hrs are recommended.

mTOR Inhibitors for Hepatocellular Cancer (HCC) - A moving Target -

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Background: mTOR (mammalian target of rapamycin) is a central regulator of cell growth and angiogenesis.
Methods: A systematic review of the current literature
Results: The mTOR pathway is activated in 40-50% of patients with hepatocellular cancer (HCC), depending on the biomarker investigated. In different models (hepatoma cell lines, implanted HCC tumors in rats), mTOR inhibitors (mTORI) were effective in reducing cell growth and tumor vascularity. Synergistic effects were observed for mTORI and chemotherapeutic agents in these studies while other combinations involving mTORI and inhibitors of growth hormones and angiogenesis are awaiting further clinical testing.
A number of mTORI are already clinically available (sirolimus, temsirolimus, everolimus), sharing similiar pharmacokinetic parameters (except for absorption) and side effects. Clinical data are yet preliminary and are mainly derived from retrospective studies in patients who had received liver transplantation for HCC. Those patients had received sirolimus thereafter for immunosuppression and a much lower rate of tumor recurrence than with calcineurin inhibitors alone was noted.
Conclusions: Current prospective trials for treatment of advanced HCC include mTOR inhibitors alone or in combination with either transarterial chemoembolisation or other systemic drugs.
Authors' disclosure statement: The author has received a research grant and consulting fees by Novartis Oncology, Nueremberg, Germany

Liquid chromatography-tandem mass spectrometry assay for determination of raloxifene and its two glucuronide metabolites in human plasma and serum

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Background: Anti-osteoporotic agent raloxifene has recently attracted a lot of attention due to the newly discovered potent breast cancer preventing effects. Unconjugated raloxifene plasma concentrations are very low, c_{max}^{ss} is only 1.36 ng/mL, while the majority of circulating raloxifene is in conjugated form with glucuronic acid. In addition, the raloxifene glucuronide standards were not commercially available. Therefore, the aim of our research was to develop a method for quantification of raloxifene (Ral) and its two glucuronides, raloxifene-6-glucuronide (M1) and raloxifene-4'-glucuronide (M2), in human plasma and serum samples.
Methods: Glucuronides (M1 and M2) were synthesized enzymatically with recombinant human UGT1A1, purified, characterized and used as authentic standards. The assay involves a solid phase extraction (SPE) procedure of 0.5 mL human plasma or serum spiked with haloperidol as internal standard on Strata X cartridges. After elution with 70% acetonitrile and 30% methanol containing 2% formic acid, the samples were dryed, reconstituted with 170 μL of initial mobile phase and injected on a Luna C18(2) 50x2.0 3μm column. The mobile phase consisted of 0.1% formic acid in acetonitrile (A) and in water (B) with a gradient from 10% to 100% A in 7 minutes. The flow was diverted to waste except from 3.9 to 6.3 min, when it entered the (+)ESI-QqQ. The following mass transitions were used for quantification [*m/z* +]: 650 → 474 for M1 and M2 and 474 → 112 and 376 → 165 for raloxifene and haloperidol, respectively.
Results: The calibration range was split into two overlapping regions with good linearity (r^2 > 0.99) covering a wide range from 0.200 to 340 μg/L, from 1.600 to 2720 μg/L and from 0.088 to 60.00 μg/L, for M1, M2 and Ral, respectively. The achieved limits of detection were 8, 11 and 6 ng/L for M1, M2 and Ral, respectively. The method was successfully applied to a pharmacogenetic study of UGT1A1*28 polymorphism on 57 postmenopausal women taking 60 mg Ral, where a significant effect of genotype on total raloxifene was observed.
Conclusion: The developed assay proved to be sensitive, accurate and precise. We expect this method can be applied with little modifications also to newer drugs from the SERM family, like bazedoxifene, arzoxifene and lasofoxifene.

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Prognostic and predictive biomarkers in thyroid diseases: "magic bullets" for a personalized therapeutic strategy

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Background: Although a malignant conversion from benign thyroid nodules is rare, in worldwide population thyroid cancer incidence has increased. The majority of thyroid cancers show a very good outcome, are well-differentiated originating from the follicular epithelium, and are subdivided into papillary and follicular carcinomas. Undifferentiated carcinomas and medullary thyroid carcinomas have a poor prognosis, arise from C cells and are less common. Prognostic evaluation of thyroid cancer is determined by biomarkers of diseases such as histological type and stage of tumour at diagnosis. However, prediction for the outcome of the cancerous disease is strongly determined by specific characteristics of the patient such as age and response to initial treatment. Therefore, different scoring systems, based on multiple regression analysis of combined predictive factors, have been proposed for stratifying patients with thyroid cancer into low and high risk prognostic groups.
Methods: In this issue a timely and detailed review of the most recent advances in prognostic and predictive biomarkers of thyroid cancers is provided, including their clinical impact for a personalized management of cancerous disease.
Results: The most used scoring system for predicting survival is the TNM staging system but other scoring systems including AMES, AGES and MACIS, the Ohio State University Scoring System are associated for a correct prognostic evaluation of thyroid cancers. Prediction of thyroid cancer outcome is commonly based on circulating thyrogobulin and calcitonin measurement in complete absence of eutopic thyroid tissue. New possible prognostic biomarkers are levels of PA system components, over-expressions of met and BRAF mutations. Expressions of multidrug resistance 2 represent new predictive biomarkers in medullary thyroid carcinoma.
Conclusions: Thyroid cancer management is based on total thyroidectomy, ablative doses of radioiodine and suppressive treatment. The follow-up is based on measuring serum thyroglobulin and imaging with radioiodine scans. New personalized treatments will use genetic biomarkers of thyroid cancer as therapeutic targets.

according to registration: Trost Jorgensen

Personalized Medicine - a Paradigm Shift in the Treatment of Cancer

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Background: The change from blockbuster medicine to personalized medicine will to a large extent influence the way that drugs are going to be developed, marketed and prescribed. In the future, it may be a pharmacodiagnostic test that decides which drug to choose for the individual cancer patient and to a less extent the average results from large randomized clinical trial combined with traditional tumor histology or the marketing efforts of the pharmaceutical companies [1].
Methods & Results: The implementation of personalized medicine will be a stepwise process where the stratification of patients, based on pharmacodiagnostic testing, into biological subgroups will be the first important step. Today, this is already the situation for several cancer diseases, e.g. breast cancer [2]. Development of pharmacodiagnostic tests are not only restricted to new drugs. As our knowledge about pathophysiology increases and the mechanisms of action of the drugs are explained, it will also be possible to develop such tests for drugs that are already on the market. One recent example is the predictive TOP2A FISH test for anthracyclin treatment in primary breast cancer. A number of clinical studies, including DBCG 89D, have shown that patients who have tumors with TOP2A gene aberrations, especially amplifications, have a significantly better effect of anthracycline-based chemotherapy than patients with a normal TOP2A gene status [2, 3].
Conclusions: Within cancer medicine, which has been on the forefront with respect to personalized or stratified medicine, it is expected that very few drugs, if any, will be prescribed without pharmacodiagnostic testing at the end of the next decade. With such development in mind other important challenges will arise such as education of health care professionals and a health care infrastructure that are able to cope with the more individualized treatments.
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Authors' disclosure statement: The author is a previously employee of Dako Denmark A/S

HIV Protease Inhibitor: An Antifungal Agent?

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Background: Protease inhibitors were shown to inhibit Candida albicans adherence to epithelial cells but not endothelial cells. Whether protease inhibitors have any effect on C. albicans adherence to acrylic surface and can be used as an antifungal is still unknown. Aims: The current study aimed to investigate whether protease inhibitors attenuate Candida albicans adherence to acrylic surface. The effect of three protease inhibitors, namely Saquinavir, Ritonavir and Indinavir on adherence were compared.
Methods: C. albicans suspensions were pre-treated with different concentrations (0.8, 4, 20, 100 and 500 μM) of Saquinavir, Ritonavir or Indinavir for one hour. The yeast cells were then allowed to adhere on acrylic strips treated with human pooled saliva for another hour (Group A). Adherence was determined by calculating the percentage of cell area over the acrylic surface using an image analyser. Another group with C. albicans not pre-treated with protease inhibitors (Group B) and a control group with no protease inhibitors added (Group C) were also included.
Results: All three protease inhibitors significantly attenuated adherence of C. albicans to acrylic surface. Group B showed significant reduction in adhesion compared with Group C. 50% reduction in adherence occurred at concentrations of 100 μM, 100 μM and 20 μM, for Saquinavir, Ritonavir and Indinavir, respectively. A dose dependent inhibition of adhesion were observed for all the protease inhibitors in Group A, which was significantly higher in Indinvavir than in Saquinavir and Ritonavir. However, such difference disappeared at concentration of 500 μM.
Conclusions: Protease inhibitor had a direct effect on C. albicans pathogenicity; it attenuated C. albicans adherence to acrylic surfaces in a dose related fashion. Moreover, different protease inhibitors exhibited different degrees of inhibition.

Expression of Topoisomerase I and IIα Protein in Primary Colorectal Cancer; is That the Culprit of Recurrent Disease Following 5-Fluorouracil-based Adjuvant Chemotherapy?

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Background: Human DNA topoisomerases I and II (topo-I and -II) are essential for vital cellular processes such as DNA replication, transcription, translation, recombination and repair. Following a chain of observations and pilot studies, we present the findings of a pioneer study, in which topo-I and -II expression was correlated with outcome after chemotherapy in primary and relapsed colorectal cancer.
Methods: Patients with colorectal cancer that had recurred, following surgery and adjuvant chemotherapy and underwent a second operation were included in the study. All had undergone surgical resection of the primary tumor and received post-operatively 5-FU-based (5FU+Leucovorin, Mayo Clinic regimen) adjuvant chemotherapy. Tumor tissue was collected at the initial operation from the primary tumor and at the time of recurrence (during the second operation following chemotherapy). All tissues samples were analyzed for levels of expression of both topo-I and topo-IIa using standard three-step immunohistochemistry on paraffin sections.
Results: Forty patients were included in the study. Levels of expression of topo-I and topo-II were higher in malignant cells from tumor recurrences compared to primary tumors (p=0.0001 for both). There was a statistically significant positive relationship between patients' age and levels of topo-I (p=0.011) and topo-II (p=0.011) expression.
Conclusions: The study results underscore the role of topoisomerase expression in colorectal cancer and suggest a potential role in tumor recurrence. This model could be further studied, to include other forms of neoplasia and infection, in an effort to elucidate the development of chemotherapy drug resistance, thus optimizing treatment strategies and improving cancer patient care.

Snake and Snail Neurotoxins – Magic Tools for Targeting Different Subtypes of Nicotinic Acetylcholine Receptors

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Background: Neurotoxins from snake venoms distinguish various nicotinic acetylcholine receptors (nAChR): α-bungarotoxin and other long-chain α-neurotoxins potently block muscle-type and neuronal α7 nAChRs, short α-neurotoxins target muscle-type nAChR, κ-bungarotoxin blocks α3β2 nAChR. Labeled α-neurotoxins are used to quantify nAChRs at different pathologies (muscle dystrophies, Alzheimer's and Parkinson's diseases, chronic pain). Some α-conotoxins, neurotoxic peptides from *Conus* snails, distinguish muscle-type nAChR, other block distinct neuronal nAChRs. However, new more potent and selective toxins are in need.
Methods: combination of HPLC and mass-spectrometry is used for isolation and structure determination of new proteins from snake venoms. Naturally occurring α-conotoxins and their analogs were obtained by solid-phase peptide synthesis. Analysis of activity of toxins was performed in binding assays with *Torpedo* nAChR membranes, α7 nAChR cell line and water-soluble acetylcholine-binding proteins (AChBPs), excellent models of the nAChR extracellular ligand-binding domain, and by electrophysiology on distinct nAChRs expressed in *Xenipus* oocytes
Results: New proteins were isolated from the *N.kaouthia* cobra venom. It was discovered that practically non-toxic weak toxin (WTX) blocks muscle-type and α7 nAChR and potently inhibits cell proliferation indicating its potential anticancer activity. For the first time a disulfide-bound dimeric α-cobratoxin was isolated and shown to interact not only with *Torpedo* and α7 nAChRs, but also with α3β2 nAChR, the latter activity found only in κ-bungarotoxin (collaboration with D.Bertrand, Geneva). Synthetic α-conotoxins bound with different affinity to AChBPs. The X-ray structure of α-conotoxin complexes with *A.californica* AChBP was determined (collaboration with A.Smit and T.Sixma, Amsterdam), unraveling the binding site architecture and providing a basis for design of novel more potent and selective antagonists and agonists of various nAChRs.
Conclusions: Thus, the presented results from our and other laboratories show that protein and peptide neurotoxins serve not only as magnifying lenses accurately disclosing the details of the nAChR binding sites, but also open the ways for design of new drugs.

Myostatin Inhibiting Peptide Works as a Magic Bullet to Increase Skeletal Muscle Mass and to Ameliorate Muscle Pathology in Muscular Diseases by Transgenic Expression

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Background: Increase of skeletal muscle mass and prevention of muscle atrophy have tremendous medical needs. Myostatin inhibition is capable of increasing skeletal muscle mass and preventing muscle atrophy. It is a promising therapeutic strategy for muscle wasting diseases such as muscular dystrophy and aging. It is also possible that myostatin inhibition will become a novel doping strategy that is difficult to detect in urine or blood tests.
Methods: Myostatin inhibitors include myostatin propeptide, follistatin, and myostatin antibodies. We have developed the myostatin-specific inhibitors derived from follistatin by domain deletion and shuffling. Transgenic mice expressing the myostatin inhibitor, called FSI-I, under the control of a skeletal muscle-specific promoter showed increased skeletal muscle mass and strength (Faseb J. 2008). We crossed FS I-I Tg with *mdx* mice, a model for Duchenne muscular dystrophy. Microarray analysis was performed.
Results: The skeletal muscles in the *mdx*/FS I-I mice showed enlargement and reduced cell infiltration. The muscle strength was recovered in the *mdx*/FS I-I mice. Our data indicate that myostatin inhibition by FS I-I has a therapeutic potential for muscular dystrophy. Microarray analysis showed the remarkable changes of expression of enzymes for fatty acid metabolism such as acetyl-CoA carboxylase (ACC) and carnitine palmitoyltransferase (CPT-1) in skeletal muscle in FS I-I Tg. Expression of molecular markers for mitochondria such as cytochrome C and uncoupling proteins increased.
Conclusions: 1) Myostatin inhibitors derived from follistatin are possible candidates of "Magic Bullets" that will increase skeletal muscle mass and may change the nature of our body composition and quality of life. 2) Microarray analysis suggests the changes of expression of metabolic enzymes. 3) Mitochondoria numbers in muscles and adipose tissues increase by myostatin inhibition by follistatin-derived molecules

Revolutionary impacts of caffeine-potentiated chemotherapy on osteosarcoma treatment

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Background: We developed innovative caffeine-potentiated chemotherapy for musculoskeletal sarcomas and good results were obtained for limited number of patients with various carcinomas. Caffeine can enhance cytocidal effects of anticancer agents in terms of its DNA repair inhibiting effect. Caffeine never allows the cell cycle delay which is mandatory for tumor cells to repair damaged DNA, leading to tumor cell death eventually. We report on a long-term result of nonrandomized prospective study about caffeine-potentiated chemotherapy and function-preserving limb saving surgery for osteosarcoma.
Methods: Fifty-one patients with non-metastatic osteosarcoma were treated with neoadjuvant chemotherapy consisting of cisplatin, adriamycin, and caffeine. Intraarterial chemotherapy was performed 3 to 5 times preoperatively and then intravenous chemotherapy 6 times postoperatively. Surgical tumor margin was reduced for chemo-responders.
Results: Total tumor necrosis was histologically demonstrated in 41 patients (80%), more than 90% tumor necrosis in 6, and no response in 4. Overall effectiveness rate was 92%. Overall 5-year survival rate was 93% and event-free survival 81% with a mean follow-up of 105 months. Lung metastases newly developed in 9 patients. Seven of those have still no evidence of disease after metastasectomy and chemotherapy. Important anatomical structures such as epiphysis, muscle, tendon, ligament, tendon, and neurovascular bundles were mostly preserved in 47 chemo-responders to improve limb function. Namely, minimizing tumor resection margin was feasible under the umbrella of caffeine-potentiated chemotherapy. Most of patients with excellent or good limb function have retuned to normal athletic activities.
Conclusions: Introduction of caffeine to osteosarcoma chemotherapy led to revolutionary advancement of survival and surgical treatment in patients with osteosarcoma. Randomized trials of caffeine-potentiated chemotherapy will provide much more clear evidence in musculoskeletal sarcomas and other cancers.

Severing the Gordian Knot: Are Glycolipids the solution for Effective Vaccines against Malaria and HIV?

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The eradication of global pathogens responsible for endemic and pandemic diseases hinges upon the development of effective vaccines. This is certainly the case for HIV and malaria. However, our inability to elicit "strong and long-lasting" protective T cell responses, particularly CD8+ T cell responses, has been a major obstacle to successful vaccine development. Accordingly, adjuvant technologies will likely be critical not only to overcome pre-existing immunity to viral vaccine vectors but also to further enhance vaccine immunogenicity. In our previous studies, a glycolipid, called alpha-galactosylceramide (alpha-GalCer), which binds to CD1d molecules and stimulates natural killer T (NKT) cells, was found to display an adjuvant activity and enhance protective CD8+ T cell responses elicited by HIV and malaria vaccines. In search of a more potent adjuvant, we have evaluated a compound library of approximately 100 new glycolipids and have identified several alpha-GalCer analogues that act as powerful NKT cell ligands. The evaluation process includes the testing of ability for glycolipids to stimulate human invariant NKT cells in vitro, as well as their ability to stimulate homologous dendritic cells (DCs). The selected compounds were then inoculated into mice in order to determine their ability to stimulate invariant NKT cells and DCs in vivo. By a series of assays, a few compounds were selected based on their potent biological activity. Finally, we have successfully identified a lead compound that displays a superb adjuvant effect against HIV and malaria vaccine platforms in a mouse model. Since CD1d molecules and NKT cells are very much conserved between mice and humans, we anticipate that the lead compound that we identified will improve the efficacy of various vaccines in humans in the near future.

Protective effects of minocycline on methamphetamine-induced dopaminergic neuronal damage: a positron emission tomography (PET) study with conscious monkeys

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Background: Previous PET studies of methamphetamine (METH) abusers suggest that psychotic symptoms may be attributable to the reduced dopamine (DA) transporters (DAT) in the human brain. To search the effective treatments, the neuroprotective effects of minocycline, a second-generation tetracycline, on METH-induced DA neuronal damages in the striatum (Str) were evaluated with animal PET with conscious monkeys. Aims: 1) To develop monkey model of impaired Str DA functions. 2) To assess the METH-induced damages of Str DA system with PET. 3) To evaluate the neuroprotective effects of minocycline on damaged Str DA system by METH.
Methods: This study included 10 male adult rhesus monkeys (Macaca mulatta,weight: 4.5 – 6.4 kg,). To induce dysfunction of Str DA system, METH (2 mg/kg, i.m.) was injected three times with 3-hours intervals, and minocycline (200 mg/kg, s.c.) was administered 0.5 hr before 1st METH injection or 0.5 hr post 3rd METH injection in 1st day, followed by minocycline administration 2 times a day from 2nd day to 7th day. PET scans with ligands of [^{11}C]2□- carbomethoxy-3□-(4-fluorophenyl)tropane (□-CFT) for presynaptic DAT and [^{11}C]SCH 23390 for postsynaptic D1 receptors (D1R) were performed on pre-treatment, 2nd and 6th days of chronic administration of minocycline (200 mg/kg) under conscious condition.
Results: Repeated METH injection drastically reduced [^{11}C]□-CFT availability for DAT in Str, while while no significant changes in [^{11}C]SCH23390 binding to D1R. Pretreatment and subsequent administration of minocycline significantly attenuated the reduction of DAT in Str of monkeys treated with METH. Furthermore, posttreatment and subsequent administration of minocycline also significantly protected the reduction of DAT. In contrast, repeated administration of minocycline alone did not alter the density of DAT in Str. These results demonstrated that minocycline protects against METH-induced neurotoxicity in the monkey brain.
Conclusions: 1) Drug abuse model of monkey was developed by repeated METH injection. 2) METH injection reduced DAT in Str. 3) Subsequent minocycline administration significantly protected the reduction of DAT.

Phase I Study of S-1 Combined with Irinotecan (CPT-11) in Patients with Advanced Colorectal Cancer

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Backgrounds: S-1 is a rationally developed combination of tegafur, a prodrug of 5-FU, 5-chloro-2,4-dihydroxypyrimidine, an inhibitor of 5-FU catabolism, and potassium oxonate, an inhibitor of 5-FU-induced diarrhea. S-1 is one of the most active single agents for outpatient treatment of patients with advanced colorectal cancer. A dose-escalation study of irinotecan (CPT-11) combined with S-1 was performed to determine the maximum-tolerated dose (MTD), recommended dose (RD) and dose-limiting toxicities (DLTs) in advanced colorectal cancer.
Methods: S-1 was administered orally at 80 mg/m^2/day for 21 consecutive days followed by a 2 week rest. CPT-11 was given intravenously on days 1 and 15 of each course, at an initial dose of 60 mg/m^2/day, stepping up to 80, 100 or 120 mg/m^2/day, depending on the DLT. Courses were repeated every 5 weeks, unless disease progression or severe toxicities was observed. The European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30 was administered at baseline and 1 week after each course to evaluate quality of life (QOL).
Results: A total of 20 patients were entered in this study. The median number of courses delivered at the RD was 6, and the mean relative dose intensity of S-1 and CPT-11 was 0.97 and 0.96, respectively. Only 1 of 14 patients at the RD needed to reduce the dose of CPT-11. Eighty-five percent of the treatment courses with the RD were delivered in the outpatient clinic. The MTD of CPT-11 was considered to be 100 mg/m^2, because 2 of 3 patients developed DLTs, anorexia and diarrhea. Therefore, the RD of CPT-11 was set at the dose of 80 mg/m^2. The overall response rate (RR) was 50% (7/14) and the RR at the RD was 55% (6/11), suggesting a promising degree of clinical efficacy. There was no significant difference in the scores of most of QOL dimensions after treatment. In general, the scores of QOL dimensions at 1 to 3 time point were not worse, and those at 4 to 6 time point were not better than those at baseline, respectively.
Conclusions: A combination of S-1 with CPT-11 can be recommended for further phase II studies in patients with advanced colorectal cancer.

Mechanism of Resistance in Non Hodgkin's Lymphoma- Expression of Fas Ligand on Endothelial Cells Lining Blood Vessels

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Background: n this study we investigated how the abnormal expression of Fas/FasL influences rituximab-mediated cell death, to establish modes of resistance in Mantle Cell Lymphoma (MCL) cells to current treatment regimens.
Methods: Xenograft models were derived following injectin of MCL Z-138 or JVM-2 cells into Rag-2M mice. Rituximab is a monoclonal antibody targeted against the B cell marker CD20 and treatment at doses of 1, 2.5, 5 and 10 mg/kg was given every other day for a total of 5 treatments in low tumor bearing mice (palpable) and high tumor bearing mice (200mg). Each treatment arm included groups of 6 mice and the study was done in triplicate. *In vivo* efficacy data was analyzed using SPSS software. The time taken for subcutaneous tumors to reach a size of 0.4 cm^3 was analyzed using Kaplan-Meier curves for survival analysis. Treatment and control groups were compared using a log-rank test. Patient samples were obtained from the BC Cancer Agency and consisted of 10 MCL, 10 Follicular Lymphoma, and 10 Diffuse Large B cell Lymphoma.
Results: In contrast to rituximab-insentive JVM-2 xenografts, tumors derived following injection of Z-138 human MCL cells were sensitive to rituximab, as judged by complete tumor regressions and decreases in cell proliferation (as measured by Ki67). Staining for FasL in Z-138 xenografts demonstrates that FasL is expressed on endothelial cells lining tumor blood vessels and was confirmed in patient samples. Coincidence of the FasL signal and CD31 targeting blood vessels was confirmed by double immunofluorescence staining on frozen sections. Expression of FasL lining tumor blood vessels led to exclusion of NK cells from the tumor microenvironment unless treated with Rituximab (as measured by immunofluorescence staining and real-time PCR). Tumor-associated macrophages were predominant in the tumor milieu and were downregulated following Rituximab treatment (as measured by immunoflorescence staining and western blot analysis).
Conclusions: 1) We describe here for the first time a novel mechanism of immune evasion in MCL and other Non-Hodgkin's Lymphomas via upregulation of FasL on endothelial cells lining tumor blood vessels.

Authors' disclosure statement: This work is currently unpublished and we trust that the data shared herein will remain confidential.

Interactions between Cytochrome P4504A, Cyclooxygenase and Nitric Oxide Synthase during Endotoxemia: Therapeutic Implications for Inflammatory Diseases

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Background: Increased production of nitric oxide (NO) and prostaglandins (PGs) associated with a decrease in formation of 20-hydroxyeicosatetraenoic acid (20-HETE) contributes to development of hypotension during inflammatory diseases such as endotoxemia. Aim: To summarize of our recent findings indicating interactions of cytochrom P450 (CYP) 4A, cyclooxygenase (COX) and inducible NO synthase (iNOS) mediate hypotension in rats treated with endotoxin (ET).
Methods: Conscious male rats received ET (10 mg/kg) or saline (4 ml/kg) at time 0. Blood pressure (BP) was measured using a tail-cuff device. Separate groups of ET-treated rats were given 1,3-PBIT (iNOS inhibitor; 10 mg/kg), indomethacin (nonselective COX inhibitor; 5 mg/kg), piroxicam (COX-1 inhibitor; 10 mg/kg), NS398 (COX-2 inhibitor; 10 mg/kg), 5,14-HEDGE (a synthetic analogue of 20-HETE; 30 mg/kg) or 20-HEDE (a competitive antagonist of vasoconstrictor effects of 20-HETE; 30 mg/kg) 1 h after injection of saline or ET. The rats were sacrificed 4 h after ET challenge and blood, kidney, heart, thoracic aorta (TA) and superior mesenteric artery (SMA) were collected for measurement of the enzyme protein levels and/or activities.
Results: ET-induced fall in BP were associated with increases in renal iNOS protein level and nitrite, 6-keto-$PGF_{1\alpha}$, PGE_2 and TxB_2 levels in the serum, heart, kidney, TA or SMA. ET decreased the renal CYP4A1 protein level/activity and the systemic and tissue levels of $PGF_{2\alpha}$. 1,3-PBIT prevented the ET-induced decrease in BP, renal CYP4A protein level/activity and increase in systemic and renal nitrite production. Indomethacin prevented the ET-induced decrease in BP, CYP4A protein level/activity, and increase in renal iNOS protein level/activity and systemic PGE_2 production. 5,14-HEDGE, piroxicam or NS398 prevented the ET-induced changes in BP and systemic and tissue nitrite and eicosanoid production. These effects of 5,14-HEDGE were abolished by 20-HEDE.
Conclusions: 1) iNOS-derived NO suppresses CYP4A expression/activity, increases the production of PGI_2, PGE_2 and TxA_2, and decreases $PGF_{2\alpha}$ levels. 2) PGI_2, PGE_2 and TxA_2, but not $PGF_{2\alpha}$, increase the iNOS protein expression/activity and decrease the CYP4A protein expression/activity. 3) 20-HETE may decrease the production of nitrite, PGI_2, PGE_2 and TxA_2, and increase the production of $PGF_{2\alpha}$. 4) Inhibition of NO and/or eicosanoid production or administration of a 20-HETE analog prevents the hypotension in endotoxemic rats.

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Quinolones and Metal Ions- Friends or Enemies?

TUREL I

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Background: Unpredictable and never ending battle between bacteria and mankind shows that diseases considered to be controlled or even eradicated are appearing again, often in new forms that are multidrug resistant. Therefore it is extremely important to completely understand the molecular mode of action of existing drugs which could help us to exploit them even more efficiently in the future. In last years quinolones are clinically amongst the most successful synthetic antibacterial agents and one of the famous members of this large family-ciprofloxacin (cfH) is a real blockbuster drug. It is known that quinolones can in general easily interact with metal ions. In one hand metal-quinolone interactions are disturbing because the absorption of these drugs is reduced (due to the formation of sparingly soluble metal complexes). On the other hand it is believed that metal ions are needed for the biological activity of quinolones. The aim of this work was to prepare metal-quinolone complexes and study their properties.
Methods: We have isolated several novel metal ion (magnesium, copper, vanadium, bismuth, europium) -quinolone complexes and characterized them by various physico-chemical techniques (X-ray structure analysis, spectroscopy (IR, NMR, UV-VIS), etc.). Biological activity of complexes was also tested (antibacterial activity, DNA gyrase activity, DNA cleavage, insulin mimetic behavior).
Results: Crystal structure of metal-quinolone complexes confirmed that bidentate (O, O`) bonding of quinolone through ring carbonyl and carboxylate oxygen atoms is predominant mode of coordination. Results have shown that antibacterial activity of metal complexes is not increased in comparison to that of free quinolones. The most interesting biological results were found for Cu-cfH complexes that are able to cleave DNA and for V-cfH complex that exerts *in vitro* insulin mimetic behavior. Interesting luminescence properties were also discovered for Eu-cfH complex.
Conclusions: 1) Certain isolated metal-quinolone complexes exert different kinds of biological activities and might be considered as potential new drugs. 2) Optical properties of europium-cfH complex might be useful for staining of tissues or for analysis of quinolones in biological samples.

Interaction between simvastatin, a HMG CoA reductase inhibitor, and grapefruit juice: in vitro characterization.

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Grapefruit juice is able to modify the pharmacokinetic parameters of many drugs. In particular, it increases oral bioavailability of simvastatin, a cholesterol lowering agent that acts by competitive inhibition of 3-hydroxy3-methylglutaryl coenzyme A (HMG-CoA) reductase. Simvastatin undergoes an important first pass metabolism and this is thought to be partly responsible for its low bioavailability after oral administration. Simvastatin is a prodrug that requires metabolic activation through hydrolysis by esterases to form the active simvastatin acid. Besides, simvastatin is a substrate for cytochrome P450 enzymes. It is also an OATP (organic anion transporter peptide) carrier substrate belonging to the solute carrier (SLC) family and MDR-1 efflux carrier belonging to the ATP binding cassette (ABC) superfamily of membrane transporters.
The objectives of our works were first to identify some constituents of grapefruit juice involved in this interaction and secondly to characterize and quantify the mechanisms of this interaction in the intestine and in the liver. We have studied two groups of compounds involved in this interaction: i) the flavonoids such as the naringin and its aglycon form, the naringenin and ii) the furocoumarins (psoralens) such as bergamottin and its metabolites. We have evaluated the effects of these two compounds (naringenin and bergamottin) on the intestinal transport and the intestinal and hepatic metabolism of simvastatin by using different in vitro models.
Our works have shown that these components of the grapefruit juice were able to modify absorption and metabolism of simvastatin by inhibition of CYP-450s (in particular CYP3A) and by modulation of ABC (MDR-1, MRP2) and OATP carriers. Thus, these constituents increased the bioavailability and plasma concentrations of simvastatin, raising its potential for adverse effects.
These results should be taken into account to adjust doses in order to avoid adverse effects and risks of rhabdomyolysis when simvastatin is co-administered with grapefruit juice.

Physiology and Pharmacology of Gonadotropin-Inhibitory Hormone

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Background: Gonadotropin-inhibitory hormone (GnIH) is an hypothalamicneuropeptide which modulates reproduction by inhibiting gonadotropin secretion from the anterior pituitary gland. GnIH can also directly inhibit reproductive behaviors, possibly via action within the brain. Identification of the distribution of GnIH neurons and fibers may provide us with clues to how the brain controls reproductive activities of the animal. We identified human and macaque GnIH peptides, and characterized the location and connectivity of GnIH neurons in the macaque brain.
Methods: 5 adult human (*Homo sapiens*) and 5 adult macaque (*Macaca mulatta*) hypothalami were used to identify the mature GnIH peptides by mass spectrometry combined with immunoaffinity purification. Location of GnIH neuronal cell bodies and fibers were identified by *in situ* hybridization and immunocytochemistry. Morphological interactions of GnIH neurons with gonadotropin-releasing hormone (GnRH)-I, GnRH-II, dopamine and β-endorphin neurons were analyzed by double labeling immunocytochemistry.
Results: We identified human GnIH (MPHSFANLPLRF-NH2 and VPNLPQRF-NH2) and macaque GnIH (SGRNMEVSLVRQVLNLPQRF-NH2) by mass spectrometry. The majority of GnIH precursor mRNA positive and GnIH-immunoreactive (GnIH-ir) cell bodies were localized in the intermediate periventricular nucleus (IPe) in the macaque hypothalamus, as determined by *in situ* hybridization and immunocytochemistry, respectively. Abundant GnIH-ir fibers were observed in the nucleus of stria terminalis in the telencephalon; habenular nucleus, paraventricular nucleus of thalamus, preoptic area, paraventricular nucleus of hypothalamus, IPe, arcuate nucleus of hypothalamus, median eminence and dorsal hypothalamic area in the diencephalon; medial region of superior colliculus, central gray substance of midbrain and dorsal raphe nucleus in the midbrain; and parabrachial nucleus in the pons. GnIH-ir fibers were further observed in close proximity to GnRH-I, dopamine, β-endorphin and GnRH-II neurons in the preoptic area, IPe, arcuate nucleus of hypothalamus and central gray substance of midbrain, respectively.
Conclusions: GnIH neurons may thus control reproductive activity of primates by regulating several neural systems in the brain in addition to inhibiting pituitarygonadotropin release.

A novel view of the pathophysiology of psychiatric disorders and development of pharmacotherapy based on brain energy metabolism

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Background: Lactate, an energy substrate for neural activity like glucose, has been shown to be produced by astrocytes under the regulation of glutamatergic tone. The serotonin (5-HT) system, specifically 5-HT_{1A} receptors, is suggested to play a key role in anxiety and affective symptoms. The aims of this study were: 1) to evaluate the effect of 5-HT_{1A} agonists on lactate production in the medial prefrontal cortex (mPFC) and basolateral amygdala (BLA); and 2) to investigate the role of glutamate transporters in the modulation of lactate production.
Methods: This study used 94 adult male Wister rats (5-7 rats per each groups, weight: 280 – 300 g). Using in vivo microdialysis technique, we measured extracellular lactate concentrations in the mPFC and BLA. Footshock stress (FS; 0.5 mA for 5 s administered every 30 s for 20 min) was administered using a plastic communication box connected to a shock-generator and a timer box.
1) Tandospirone (2.0 mg/kg), a partial agonist at 5-HT_{1A} receptors, and WAY-100635 (0.5 mg/kg), a selective 5-HT_{1A} antagonist, were administered intraperitoneally 10 min and 40 min respectively before the start of FS.
2) Dihydrokainate (DHK), a glutamate uptake inhibitor, was dissolved in artificial cerebrospinal fluid (CSF). Artificial CSF containing DHK was perfused at a rate of 5.0 µl/min into the dialysis probe. Twenty minutes after the start of DHK (0.1 mM) perfusion, FS was administered.
Results:
1) Tandospirone attenuated the FS stress-induced increase of eLAC in both of the brain regions, which was blocked by pretreatment with WAY-100635.
2) DHK also attenuated stress-induced increment of eLAC in the mPFC, and completely prevented it in the BLA.
Conclusions: The results of this study indicate 1) FS stress-induced increase in lactate production is partly regulated by 5-HT_{1A} receptors both in cortical and limbic regions, 2) glutamate transporters regulate lactate availability in astrocytes, and that 3) rapid energy demand induced by glutamate contributes to local lactate production. Research into interactions between neurotransmitters and lactate metabolism may provide a novel view of the pathophysiology of some stress-related disorders, e.g., mood disorder, anxiety disorder, and post-traumatic stress disorder.

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Antitumor potency of angiotensin II receptor blocker for prostate cancer

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Since a low prevalence of cancer in hypertensive patients receiving angiotensin converting enzyme inhibitors was reported, a biological action of angiotensin II (Ang-II) on the development or progression of cancer has been drawn attention. It is known that Ang-II plays a fundamental role not only as a vasoconstrictor in controlling blood pressure and electrolyte/fluid homeostasis, but also as a mitogenic factor through the Ang-II type-1 (AT1) receptor in cardiovascular cells. In the last decade, a widespread use of AT1 receptor blockers (ARBs), antihypertensive drugs, has contributed more attractive information on the involvement of Ang-II in cancer. Interestingly, there is increasing evidence that the renin-angiotensin system (RAS) is implicated in the development of various cancers.
To date we have reported that prostatic RAS related to cell proliferation and angiogenesis has the potential especially in carcinogenesis of prostate and ARBs can suppress them by inhibiting signal transduction pathways or angiogenesis through AT1 receptor. Clinically, we demonstrated the administration of ARBs for hormone refractory prostate cancer patients induced the decline and stabilization of serum prostate specific antigen (PSA) with an improvement in performance status. Also, we confirmed that the deceleration of PSA-doubling time by ARB treatment was shown in patients with PSA failure after radical prostatectomy. Our in vitro study demonstrated that Ang-II up-regulated oxidative stress-related proteins and enhanced the production of O_2^- radical in prostate cancer cells, and conversely ARBs inhibited them.
From basic and clinical data, we believe that ARB has a novel ability to decelerate PSA elevation and suppress the incidence of prostate cancer, which implies that ARB may have a chemopreventive activity for prostate cancer.

The critical role of IL-15 *trans*-presentation in the antitumor effects mediated by the combination therapy with Imatinib and IL-2

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Background: The synergistic antitumor effect of the combination therapy with imatinib mesylate (IM) and IL-2 has been shown to depend on NK1.1 expressing cells and led to the first description of an important effector cell population called Interferon producing killer dendritic cells (IKDC). IKDC were initially described as $CD11c^{int}B220^{+}NK1.1^{+}$ tumor infiltrating cells that mediated the antitumor effects of combination therapy with IM and IL-2 in B16F10 melanoma-bearing mice (Taieb et al. Nat. Med. 2006). This work aims at further investigating the mechanism involved in antitumor capacity of IKDC in this combination therapy.
Methods: We used a systemic mouse B16F10 melanoma model in C57Bl/6 mice and different transgenic mice. 3 x 10^5 B16F10 cells were injected at day 0 into the tail vein of mice. 200µl IM (Gleevec®, Novartis) 150mg/kg was given orally twice a day combined with IL-2 (1 x 10^5 IU of rhIL-2, twice a day i.p.) from day 0 to day 10 after tumor inoculation. Control groups received H_2O+PBS, IM or IL-2 alone. At day 12, mice were sacrified and lung metastasis were enumerated.
Results: Here, we show that the antitumor efficacy of the combination therapy was compromised in IL-15 and IFN type 1R loss-of-function mice. IL-15R□ was required for the proliferation of IKDC during therapy with IM and IL-2. *Trans*-presentation of IL-15 by IL-15R□ activated IKDC to express CCR2 and to respond to type 1 IFN by producing CCL2. Moreover, the antitumor effects of the combination therapy correlated with a CCL2-dependent recruitment of IKDC into tumor beds.
Conclusions: Our data indicate that the IL-15 driven peripheral expansion and intratumoral chemoattraction of IKDC is an important factor for the immunomodulatory effects of IM+IL-2.
Since our data suggested that the combination therapy IM+IL-2 could be useful to boost natural immunosurveillance against tumors sensitive to TRAIL-dependent apoptosis, we launched the Phase I trial "IMAIL-2" that aims at targeting Imatinib-resistant and TRAIL sensitive cancers. It is conceivable that the monitoring of innate effectors in patients treated with high dosages of IL-2 combined with Imatinib might allow the identification of the human counterpart of mouse IKDC.

Designing Bone-Seeking Proteins

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An ideal therapeutic agent for bone diseases should act solely on bone tissue with no
pharmacological activity at other anatomical sites. Current therapeutic agents, however, do not usually display a preferential affinity to bones and non-specifically distribute throughout the body after administration. Attempts to design bone-specific agents have relied on engineering a desired therapeutic agent with bone-seeking molecules so that the latter delivers the therapeutic agents specifically to bones. In this presentation, we will summarize the latest attempts to engineer bone-seeking therapeutic agents based on formulating therapeutic agents with bisphosphonates, a class of compounds with high affinity to biological apatite. We first provide a relevant summary of the structure of bone mineral and bisphosphonates, highlighting the mode of interaction between these two entities. A summary of recent attempts to formulate bisphosphonates with traditional therapeutic agents to restrict their activities to bone tissues is then provided, with special emphasis on the structure-function relationships of the engineered compounds. Finally, attempts to use bisphosphonates to deliver macromolecular therapeutics (i.e., proteins) are summarized, based on recent data from the authors' lab. The collective research into bone-seeking medicinal agents is progressively laying the foundation for next-generation 'magic bullets' that display desirable activities at the disease sites with no undesirable activity on other organ systems.

Chemotherapy of Duchenne's muscular dystoropy

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Background: Duchenne muscular dystrophy (DMD) is a X-linked muscular abnormality caused by the loss of dystrophin and is one of the most gravely genetic disorders. We have recently found that hematopoietic prostaglandin (PG) D synthase (H-PGDS) was induced in grouped necrotic muscle fibers in DMD patients (Okinaga T. et al., Acta Neuropathol., 104, 377-384 (2002)). Cyclooxygenase-2 and phospholipase A_2 were also synchronously induced in the H-PGDS-expressing necrotic muscle fibers. We developed novel H-PGDS inhibitors based on the X-ray crystallographic analysis of human H-PGDS complexed with its prototype inhibitor, HQL-79 (Aritake K. et al., J. Biol. Chem., 281, 15277-15286 (2006)). In this study, we investigated pathological significance of H-PGDS in a dystrophin null *mdx* mouse model and developed a novel therapy for DMD by inhibition of H-PGDS.
Methods: Localization of H-PGDS was examined in the dystrophic muscle fiber in a dystrophin null *mdx* mouse model by immunofluorescence staining with anti-mouse H-PGDS antibody. H-PGDS inhibitors were orally administrated to *mdx* mice for 5 days. The necrotic muscle in *mdx* mice was continuously measured by X-ray computed tomography (CT) imaging enhanced by non-ionic contrast media.
Results: H-PGDS was localized in the necrotic muscle fibers and accumulated macrophages in *mdx* mice. Oral administration of H-PGDS inhibitors prevented the expansion of muscular necrosis in a *mdx* mouse model and decreased the expression of mRNAs of pro-inflammatory cytokines. These results demonstrate that PGD_2 produced by H-PGDS plays important pathological roles on the expansion of muscle necrosis. H-PGDS inhibitor also accelerated the accumulation and activation of macrophages in the necrotic area.
Conclusions: These results indicate that PGD_2 produced by H-PGDS is involved in the expansion of muscle necrosis in DMD and that inhibition of H-PGDS is a novel therapy for DMD.

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Inhibition of Neutrophil Granule Exocytosis by a Novel Cell-Penetrating SNAP23 Fusion Protein: A Potential Magic Bullet?

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Background: Neutrophils are the primary cellular component of innate immunity. These cells contain four granule subsets that undergo exocytosis in response to stimulation. The role of exocytosis in mediating neutrophil activation is unknown. The hypothesis that exocytosis contributes to specific neutrophil functional responses was tested.
Methods: To inhibit exocytosis, fusion proteins containing the TAT cell permeability sequence and either the amino terminus SNARE domain of SNAP-23, (TAT-SNAP-23) or an inactive amino acid sequence (TAT-Control) were expressed. Transduction of TAT-SNAP-23 into neutrophils was confirmed by confocal microscopy. Exocytosis of secretory vesicles, specific granules and azurophil granules was determined using flow cytometry to measure the expression of CD35, CD66b and CD63, respectively.
Results: TAT-SNAP-23 inhibited the fMLP-stimulated increase in CD35 and CD66b in a concentration-dependent manner, but had no effect on the increase in CD63 in the presence of latrunculin A. TAT-SNAP-23 (5 µg/ml) reduced the stimulated increase in CD35 and CD66b expression by 90% ± 7% and 75% ± 10%, respectively. Exocytosis of gelatinase granules, measured by release of gelatinase, was reduced by 60% ± 10%. TAT-Control had no effect on exocytosis of any of the four granule subsets. TAT-SNAP-23 (5 µg/ml) had no effect on fMLP-stimulated superoxide release or bacterial phagocytosis; however, phagocytosis-stimulated H_2O_2 production was significantly reduced by 50%. Additionally, 5 µg/ml of TAT-HA-SNAP-23 significantly reduced chemotaxis, but not chemokinesis, across a transwell membrane.
Conclusion: In conclusion, a fusion protein containing a cell penetrating peptide and a SNARE domain of SNAP-23 inhibits exocytosis of three of four neutrophil granule subsets. The data show that granule exocytosis contributes to neutrophil chemotaxis and phagosomal respiratory burst activity, but not bacterial phagocytosis or respiratory burst activity stimulated by plasma membrane receptors.

Principal parallels between Interferon type I- and small inhibitory (si)RNA-based therapies

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Background: RNA interference (RNAi), a simple regulatory mechanism of gene expression and virus replication, was evolutionary replaced by the more complex interferon type I (IFN I) system. IFN I system is the major component of the innate immune response against viruses and part of the initial body defence against a range of microorganisms. However, it is less specific and it appears to be less efficient than RNAi against viruses. Here we will provide parallels between these two systems and discuss their advantages and disadvanteges for the treatment of viral infections.
Methods: In this study primary cultures of peritoneal macrophages (mΦ) isolated from congenic flavivirus susceptible or resistant mice were used as a model to study various antiviral treatments against West Nile (WN) virus. The extent of antiviral effect of treatments with IFN I, polyinosinic/polycytidylic RNA (pIC) and several off-target (random) small inhibitory (si) RNAs was quantified by virus titration while the presence/absence of viral RNA was confirmed by RT-PCR.
Results: Untreated mΦ from both susceptible and resistant mice were similarly permissive to WN virus infection although cells from resistant mice showed an initial 14% inhibition of virus growth. Following the priming with various antiviral agents, including the major antiviral cytokine IFN I, mΦ from resistant mice developed 2-4 stronger antiviral responses than cells from susceptible mice. However, a treatment with off-target siRNAs resulted in a complete eradication of WN virus replication in cells from both susceptible and resistant mice.

Treatment	Susceptible mΦ Virus titre ($log_{10}TCID_{50}$ units/10^5 cells)	*Percent inhibition (%)	Resistant mΦ Virus titre ($log_{10}TCID_{50}$ units/10^5 cells)	*Percent inhibition (%)
None	5.8	0	5.0	14
IFN I	4.9	16	2.0	66
pIC	3.2	45	0.8	86
siRNA	0	100	0	100

*Percent inhibition relative to untreated mΦ from susceptible mice
Conclusions: 1) Cells from resistant mice responded better to conventional antiviral treatments with IFN I and pIC than cells from susceptible mice. 2) Treatment with off-target siRNAs elicited the strongest antiviral effect in both cell types suggesting involvement of a resistance-unrelated mechanism. 3) Antiviral therapy based on small RNA may prove more efficient and direct than therapy based on IFN I.

WHEN SHOULD WE ADMINISTER POSTOPERATIVE INTRA-PERITONEAL MITOMYCIN THERAPY?

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Background: There is controversy about the effect of the timing of intraperitoneal administration of chemotherapeutic agents on the healing of intestinal anastomosis. We have investigated the effect on intestinal wound healing of mitomycin-C administered at different times post-operatively.
Methods: Eighty-four Wistar-Albino female rats underwent ileal resection and end-to-end anastomosis. The rats were randomly selected for intraperitoneal administration of mitomycin-C or saline as follows: mitomycin-C group (n = 65), 2 mg/kg mitomycin-C; control group (n = 13), 10 ml saline. The former was sub-divided into 5 equal groups (A 1-5) and mitomycin-C was administered postoperatively as follows: day 0 (A1), day 3 (A2), day 5 (A3), day 7 (A4) and day 10 (A5). All the rats were sacrificed on the 14th postoperative day and anastomotic bursting pressures and tissue hydroxyproline levels were determined.
Results: Five of the animals died postoperatively: 2 (15.4%) in group A1, 2 (15.4%) in group A2 and 1(7.7%) in group A3. Non-lethal anastomotic leakage was observed in a further five animals: 1 in group A1, 2 in group A2, 1 in group A5 and 1 in the control group. Groups A1 and A2 had significantly lower anastomotic bursting pressures than the other groups (p was <0.05 for each comparison). The anastomotic bursting pressures of group A3, A4 and A5 were comparable with those of the controls (p was >0.05 for each comparison). Tissue hydroxyproline levels in group A1 and A2 were significantly lower than in the controls (p values were <0.05 for each comparison) or the other mitomycin-C sub-groups (p was <0.05 for each comparison).
Conclusıons: Intraperitoneal chemotherapy impairs intestinal wound healing when applied before the 5th postoperative day. Additional therapeutic approaches are needed to prevent this potentially lethal side effect of early intraperitoneal mitomycin-C administration.
Authors' disclosure statement: This study has been published BMC Cancer as "the optimal starting time of postoperative intraperitoneal mitomycin-C therapy with preserved intestinal wound healing".

Commercialization of Recombinant Human Epidermal Growth Factor – A Nobel Prize Winning Molecule with Diverse Therapeutic Applications

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Background: Growth Factors are a distinct class of signaling proteins that modulate wound healing at a molecular and cellular level. One such Growth Factor is the Epidermal Growth Factor (EGF), a 6.2 kDa protein, whose discovery by Prof Stanley Cohen got him the Nobel Prize along with Dr Rita Levi-Montalcini in 1986. The role of EGF has been extensively investigated in normal and pathological wound healing and EGF based formulations are recently gaining therapeutic importance in wound management. Such Formulations, for obvious reasons, are developed using recombinant human Epidermal Growth Factor (rhEGF). This Presentation reviews the various approaches adopted to produce rhEGF on a large scale including the work carried out by the Authors' Group. We have been successful in producing rhEGF at a commercial level through a cost-effective, novel process yielding >99% re-natured active protein and high specific activity. The Presentation also discusses the excellent clinical results obtained by the Group with rhEGF based formulations for various therapeutic applications along with attempts to understand the mechanism of EGF at a bio-molecular level in wound healing. In the Authors' view, such a wide range of clinical studies are the first comprehensive studies in literature. Finally, the Presentation also describes the recent focus of research in new drug delivery systems that are able to protect and stabilize EGF, which is readily degraded in any chronic wound environment. It is predicted that such work would lead to future therapeutic options with Growth Factors in a diverse array of applications.
Methods: Manufacture of rhEGF & Formulations thereof: Several studies have been reported earlier on cloning in *E.coli* and yeast system for the expression of recombinant human EGF (rhEGF) and purification by RP-HPLC and Solid Phase Extraction or Expanded Bed Adsorption (EBA) Chromatography. A serious limitation of most of these studies is the final yield (<100 mg/L) and the purity obtained (<85%), thus limiting the commercial potential of such work. In our objective towards commercialization of rhEGF, we have expressed rhEGF as a tripartite tag protein consisting of N terminal TrpE sequence and C terminal with six arginine residues attached to the human EGF sequence. This fused gene was cloned in pET11b vector under the control of the T7 promoter and the protein was expressed in *E.coli* BL21(DE3)RIL strain. Fermentation conditions were optimized to express the protein as an inclusion body which was estimated around 500mg/L, and the yield obtained after downstream purification was about 400mg/L. Some of the novel aspects of our work include reduced fermentation time, lesser number of downstream purification steps and better control on refolding using EBA chromatography. The 6.2kDa protein was characterized for physico-chemical properties by RP- HPLC, Size exclusion chromatography, MALDI-TOF, Circular Dichroism, N-terminal sequencing and the biologically functionality was assessed by ELISA using functional monoclonal antibodies and 3T3 Cell lines. rhEGF Based Therapeutic Formulations: Extensive Formulation development work (both Cream and Gel based) has been carried out to arrive at the most suitable and stable Formulations at 3 different rhEGF concentrations for various applications. Safety of such Formulations has been evaluated by in-depth toxicological studies.
Clinical trials for various therapeutic indications: Three most widely investigated applications include Diabetic Foot Ulcers, Skin Grafts and Burns and these have given extremely encouraging results. Other indications for which studies have been completed include Venous Leg Ulcers, Corneal Ulcers and GI Tract ulcers. This Presentation would highlight the wound healing duration and wound size reduction results obtained with our licensed formulation REGEN D, currently under usage in India and several other countries.
Results: rhEGF has been manufactured at 100 L fermentation scale and the purified rhEGF showed a specific activity of 5 x 10^5 IU/mg protein, in comparison with NIBSC standard (Ist International Standard of rDNA-derived EGF, Code 91/530).In all clinical trials, REGEN D was found to result in healthy granulation and stimulate epithelization. Collagen levels increased significantly in REGEN D treated groups while, as is desirable, MMP-9 (Matrix Metallo Proteinases) expression got reduced. Similar encouraging results were obtained in other studies aimed at exploring the diverse range of treatment modalities available with REGEN D.
Conclusions: This Presentation describes the successful large scale manufacture of rhEGF and formulations based on rhEGF have shown great promise for enhanced wound healing in diverse cases involving diabetic foot ulcers, 1st and 2nd degree burns, bed sores, venous leg ulcers etc. This has allowed a new perspective to wound healing research involving rhEGF. Using nano-medicine drug delivery technologies, new vistas could be opened targeting enhanced stability of EGF. It is our endeavour to put EGF on the global Biotechnology map and we sincerely hope that EGF would become a potential "Magic Bullet" candidate.

Prevention of hepatotoxicity in patients undergoing anti tuberculosis treatment: A novel integrative approach

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Introduction: Conventional anti-tuberculous treatment (ATT) containing Isoniazid, Rifampicin and Pyrazinamide, is hepatotoxic: the incidence varying between 4-11%. Stopping ATT and restarting it once the enzymes are normalized are only measures practiced leading to drop-outs, incomplete treatment and recurrence in addition to occasional severe liver failure requiring liver transplant. Ayurvedic herbs like Curcuma longa and Tinospora cordifolia have shown hepatoprotective and immunostimulatory properties in rodent model of ATT induced hepatotoxicity, so a prospective two armed RCT was carried out to evaluate their potential in humans.
Methods: Patients with active tuberculosis diagnosis were randomized to a drug control group (n=192) and a trial group (n=316) on drugs plus an herbal formulation. Isoniazid, Rifampicin, Pyrazinamide and Ethambutol for first two months followed by continuation phase therapy excluding Pyrazinamide for 4 months comprised the anti-tuberculous treatment. Curcumin enriched (25%) CL and a hydro-ethanolic extract enriched (50%) TC 1 g each divided in two doses comprised the herbal adjuvant. Hemogram, bilirubin and liver enzymes were tested initially and monthly till the end of study to evaluate the result. The results were analyzed by Chi square test. ($P \leq 0.05$)
Results: Incidence and severity of hepatotoxicity was significantly lower in trial group (incidence: 27/192 *vs* 2/316, $P < 0.0001$). Mean Aspartate transaminase (AST) (195.93 ± 108.74 *vs* 85 ± 4.24, $P \leq 0.0001$), Alanine Transaminase (ALT) (75.74 ± 26.54 *vs* 41 ± 1.41, $P \leq 0.0001$) and Serum Bilirubin (5.4 ± 3.38 *vs* 1.5 ± 0.42, $P \leq 0.0001$). A lesser sputum positivity ratio at the end of 4 wk (10/67 *vs* 4/137, $P = 0.0068$) and decreased incidence of poorly resolved parenchymal lesion at the end of the treatment (9/152 *vs* 2/278, $P \leq 0.0037$) was observed. Improved patient compliance was indicated by nil drop-out in trial *vs* 10/192 in control group ($P \leq 0.0001$)
Conclusion: 1.The adjuvant herbs showed strong hepatoprotective activity 2. Improved outcome with higher and quicker sputum negativity may be due to improved patient compliance in addition to immunostimulatory action. 3 The result carries utmost significance for mal-nourished, alcoholic and immuno-compromised patients.

New Drug Development Outside of G8: Ready, Shoot, Aim

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Background: Clinical assessment in late-stage drug development should be guided by results of comprehensive preclinical safety assessments and rigorous clinical pharmacology programs. In South America (SA), new drug development is generally conducted at public universities with the support of local pharmaceutical companies willing to participate in a government-supported research project. However, preclinical studies are seldom conducted in SA universities, thereby frustrating talented investigators and industry when promising discoveries receive the envitable rejection from regulatory agencies within the developed world.
Observations: Based on experience gained over 10 years at the U-Chile where we established the first good laboratory practices (GLP) laboratory to perform preclinical studies in bacterial or animal systems in Chile. We conduct the Mammalian Erythrocyte Micronucleus test and Bacterial Reverse Mutation Assay for each substance, strictly adhering to FDA and OECD guidelines. Bacterial strains, the S9 microsomal fraction, cofactors, and other chemicals are certified and imported from the country of origin (USA or Japan). The mouse strain was originally certified and imported from CLEA-Japan and maintained in specific pathogen free (SPF) mouse facilities in our research center.
Less than 10 companies requested mutagen assays under the local regulatory authorities, and only a few persevered. The results of the assays in two products which had excellent clinical results in humans, however, were positive to the micronucleus and the Ames test.
Conclusions: 1) We recommend mandating preclinical studies in animals and bacterias according to GLP guidelines for drug approval in SA. 2) Educate local pharmaceutical companies to the impact of the mutation assay results for chemicals or drugs that are currently going into the approval process.
Authors' disclosure statement: Authors are on full-time faculty at the Universidad de Chile-Santiago. Implementation of the Genotoxic Risk Assessment Center (CERIG) was financed by a grant for development of sciences and technology FONDEF-Conicyt-Chile. The mouse facilties were implemented, in part, with a grant for the Ministery of Education of Chile, MECESUP to the Dental School, and by the Japan International Cooperation Agency (JICA). Drs. Valdivla and Kato are principals in the Japan Food Safety Center based in Santiago, Chile

Lactoferrin Acts against Infection and Inflammation through its Influence on Systemic Iron Homeostasis

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Background: Inflammations and infections are often associated to hypoferremia. Hypoferremia is an important signal of disorders of hepcidin levels which regulate the entry of iron into plasma through ferroportin (FPN), the only known cellular iron exporter. The resulting iron-overload in secretions and cells increases host susceptibility to infections. Therefore, the regulation of systemic iron homeostasis is critical to human health. We have demonstrated that lactoferrin (Lf) can modulate systemic iron homeostasis through the decrease of serum IL-6, key molecule in hepcidin and FPN synthesis. Aims: 1) To compare the effects of ferrous sulphate and lactoferrin (Lf), orally administered, in hypoferremia 2) To verify the relationship among the increase of total serum iron concentration and the decrease of host inflammation and susceptibility to infections.
Methods: The clinical trial on the therapeutic effect of Lf on systemic iron homeostasis included 171 subjects, suffering of hypoferremia. Subjects were randomly divided in two groups. The first group received an oral administration of 520 mg of ferrous sulphate, once a day (156 mg as elemental iron); and the second group received orally 100 mg of Lf, twice a day (8.8 mg as ferric iron). Haematological values and IL-6 concentration were assessed on venous blood.
Results: Ferrous sulphate administration often failed to exert significant effects on hypoferremia. Conversely, Lf highly increased the values of total serum iron and serum ferritin already after 30 days of therapy (mean values from 45 to 95 µg/dL and from 12 to 27 ng/ml, respectively). Moreover, Lf administration modulated systemic iron homeostasis by decreasing serum IL-6 concentration (mean values from 32 to 10 pg/ml), while ferrous sulphate exhibited an opposite effect.
Conclusions: 1) Lf exerted a potent effect in restoring the iron transport from cells into circulation through the decrease of serum IL-6 concentration which in turn modulates hepcidin and FPN synthesis. 2) In contrast to the administration of ferrous sulphate, Lf oral administration did not result in any side effect. 3) The capacity of Lf to decrease IL-6 leading to the rescue of haematological parameters could represent a novel therapeutic alternative in reduction of host inflammation and susceptibility to infections.

Molecular Characterization of Antibiotic Resistance in Selected Enteropathogens Isolated from Raw Food Samples in Vietnam

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Background: The emergence of antibiotic resistance in bacteria has become a serious problem worldwide. This study aims to determine the molecular characteristics of antibiotic resistance in enteropathogens isolated from raw food samples in Vietnam.
Methods: Raw food samples (n=180, comprising meat, poultry and shellfish) were collected from Ho Chi Minh City, Vietnam for the isolation of Salmonella spp., Escherichia coli and Vibrio parahaemolyticus. The isolates were tested for antibiotic resistance against 15 commonly used antibiotics by the disk diffusion method. They were further examined for the presence of mobile genetic elements conferring antibiotic resistance. Transfer of antibiotic resistance phenotypes was studied by conjugation. Salmonella genomic island 1 (SGI1) antibiotic resistance gene cluster was investigated using PCRs, Southern blot analysis and sequencing.
Results: E. coli and Salmonella spp. was isolated in 60.8% and 48.9% of the samples respectively. V. parahaemolyticus was present in 32% of shellfish samples. There were high antibiotic resistance frequencies in E. coli and Salmonella spp. isolates, especially to tetracycline, ampicillin, nalidixic acid, streptomycin, and sulphafurazole. E. coli also showed high resistance to trimethoprim (43.4%) and chloramphenicol (51.5%). Multi-resistance, i.e. resistance to at least three different classes of antibiotics, was detected in 61.6% E. coli and 20.9% Salmonella isolates, including potential human-pathogenic Salmonella serovars. V. parahaemolyticus isolates were uniformly resistant to ampicillin. Integrons harbouring genes responsible for resistance to aminoglycosides, ampicillin, trimethoprim and chloramphenicol were found in 57% E. coli and 13% Salmonella spp. isolate. Plasmids were detected in all tested isolates, many of them were larger than 95 kb. Antibiotic resistance phenotypes were found to be transferable among the isolates. SGI1 was identified in Salmonella serovar Albany isolated from chicken meat.
Conclusions: The results indicate that raw foods of animal origin in Vietnam are potential reservoirs for multi-resistant pathogenic organisms which contain a pool of mobile genetic elements, raising the awareness of antibiotic resistance in food pathogens.

One target, two bullets : from erythromycin to telithromycin, what makes the difference ?

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Background: the increasing threat of bacterial resistance and the progress of modern pharmacology stimulate the search for antibiotics capable of bypassing existing resistance mechanisms and presenting an optimized pharmacological profile.
Methods: overview of the rational design of telithromycin (TEL), as first clinically-used ketolide and semi-synthetic derivative of the macrolide erythromycin (ERY), in relation with its novel pharmacological profile.
Results:
SAR: TEL differs from ERY by (a) removal of the cladinose at position 3 and its replacement by a keto-group, (b) incorporation of an 11,12- cyclic moiety, and (c) addition of a heteroaryl-alkyl side chain. TEL can therefore interact with 2 distinct binding sites in the 23S rRNA of the ribosome. This increases its activity against ERY-S strains and maintains activity towards ERY-R S. pneumoniae with ribosomal methylation. TEL is also a poor inducer of methylase expression and less susceptible to efflux in S. pneumoniae.
PK: TEL shows an improved PK profile, with high oral bioavailability (improved acid stability), penetration in tissues and fluids, accumulation within eucaryotic cells and poor recognition by eucaryotic efflux transporters (P-glycoprotein), prolonged $t_{1/2}$ allowing for daily administration.
PD PK/PD considerations suggest optimal efficacy for isolates with MICs $\leq$ 0.25 mg/L. As ERY, however, TEL is bacteriostatic in vitro and poorly active in models of intracellular infections despite its high cellular accumulation.
Safety: TEL causes less gastro-intestinal side effects and is a less potent inhibitor of CYP3A4 than ERY. It causes however rare but serious side effects (hepatotoxicity, respiratory failure in patients with myasthenia gravis, visual disturbance, risk of QTc prolongation).
Conclusions: this story tells us how knowledge of targets helps in obtaining more powerful bullets. Further optimization of PD and safety is still needed to get "the" magic bullet acting on bacterial ribosomes.

Passive Immunization for the Protection of our Global Society against Emerging Infections

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Background: New viral diseases, like the Severe Acute Respiratory Syndrome (SARS), are an enormous threat to our society. With more than 50% of the global population living in cities and 2 billion airplane passengers per year, there is no way to escape from contact with infected person once a pandemic starts. The World Health Organisation (WHO) devoted the World Health Report 2007 to this topic. WHO concludes that there will be another Ebola or SARS sooner or later. Vaccination would be the preferred way to protect the population, but the development of a new vaccine takes about 10 years. Therefore serum therapy is a serious candidate for the therapy and post exposure prophylaxis of new viral.
diseases
Analysis: There are several powerfull approaches to generate and produce humanized or human monoclonal antibodies. But production in mammalian cell lines in bioreactors is still an expensive process and at this moment there is insufficient production capacity to produce the required quantity of antibodies.
Solutions: Innovation in production technology is necessary. One solution could be to use plants for the production of the monoclonal antibodies. Another way to solve the problem would be to make more potent neutralizing antibodies than can be administered at lower concentrations. In view of the importance of protection of the global population and the lack of alternatives, a task force for the necessary innovation is needed.

Pharmacogenetic screening and drug susceptibility and fetal malformations, past, present, and future

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Background: An understand of drug susceptibility and its relationship to possible birth abnormalities will become increasingly important in areas of clinical management for physicians of the future, particularly those in OBGYN, Pediatrics, Environmental Medicine, and Toxicology.
Aims: 1) Review past published journal articles dealing with drug susceptibility
2) Review recent advances in pharmacogenetics and analyze them for potential significance for future medical practice.
Methods:
- Reviewed our past journal publications on fetal phenytoin and other fetal drug exposures with emphasis on folate metabolism, clefting defects, CHD, and neural tube abnormalities
- Reviewed our past published literature reviews on fetal anticonvulsant exposure
- Completed literature search of the last 10 years (1997 to 2007) on new research in drug susceptibility to fetal drug exposure and birth defects.

Results:
- Identified significant past reviews of fetal drug exposure and susceptiblitly to birth abnormalities
- Identified 68 articles in the literature in the last 10 years that dealt with newborn screening, fetal abnormalities, and interventions that may lead to prevention of or decrease in some birth abnormalities.

Conclusions: New molecular genetics techniques such as identification of SNPs, candidate genes, advances in computer hardware, software, and statistical techniques have aided our understanding of genotype, drug susceptibility, and variability in clinical phenotype. Antenatal screening for metabolic abnormalities, hemoglobinopathies, hearing, and a few genetic disorders has become routine in some states in the USA and other countries. Antenatal screening has four main objectives:
- Education of parents with expanded opportunities for parental choice
- Allow for potential fetal treatment or for immediate postnatal treatment
- Allow for informed decisions regarding present and future pregnancies
- Expand the potential for improved outcomes.

However, there are multiple variables involved; many are probably still unrecognized. Fetal drug/maternal drugs screening for clinical management remains on the horizon of the future

Differences in Plasma Levels of Risperidone: Causes and Consequences

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Background: Risperidone is an atypical antipsychotic, available in various formulations. It is rapidly absorbed after oral administration and undergoes extensive metabolism. It is generally assumed that rapidly dissolving oral formulations are equivalent to oral solutions and a previous publication shows bioequivalence between an oral risperidone solution and risperidone tablets. When administration of two immediate release oral formulations results in equivalent plasma concentration profiles, essentially similar concentrations at the site of action and therefore similar efficacy and safety can be assumed.
Methods: The bioavailability of the generic risperidone solution containing sorbitol and the 1 mg immediate release originator tablet formulation was compared in 32 healthy volunteers using a standard crossover design. Both formulations contained 1 mg risperidone per dosing unit. The solution was administered using a standard dosing device to measure 1 ml that was further diluted in a glass of water.
Results: Plasma levels for the 9-hydroxy metabolite of risperidone were within bioequivalence limits. However, bioequivalence could not be claimed for the parent compound risperidone.
There are several potential explanations for the observed differences in plasma levels of risperidone. Risperidone pharmacokinetics, assay result, dosing device, excipients, design of bioequivalence study and life habits can all contribute to differences in plasma concentrations. The potential impact of these variables on daily practice will be discussed.
Conclusions: Bioequivalence between the studied 1 mg/ml generic risperidone solution and the 1 mg immediate release originator tablet was not proven in this study.
Differences in plasma levels can result in lack of therapeutic efficacy, which can have dramatic consequences in schizophrenic patients.

Antibody polyreactivity and ill-defined antigen mimicry hamper the search for effective peptide-based vaccine immunogens

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The regions of antigens that are recognized by antibodies are called epitopes while the regions of antibodies that recognize epitopes are called paratopes. Most epitopes of proteins are called discontinuous epitopes because they arise from between two and five separate segments of the polypeptide chain,that are brought together by the folding of the chain. These epitopes exist only because the chain acts as a scaffold and if the scaffold is perturbed, the epitope ceases to exist. It is not possible to isolate such epitopes independently of the rest of the protein in which they are embedded in order to show that they possess binding activity on their own. It has also not been possible so far to predict them or to reconstruct them by synthesis since this would require assembling atoms which in the protein are not held together by internal chemical bonds.
Most residues at the surface of a native protein contribute atoms to a large number of overlapping epitopes recognized by different antibodies. No clear boundaries exist between neighbouring epitopes which together form a series of antigenic sites and it is only because Mabs are used as analytical tools that protein antigenicity appears to be located in discrete epitope regions rather than in an antigenic continuum. Unfortunately the strategy of focusing on individual epitopes has been detrimental to the search for effective vaccine immunogens.
Proteins are also said to possess continuous epitopes, defined as linear protein fragments of 5 - 15 residues that are able to bind to antiprotein antibodies. Many of the residues of such continuous epitopes are not present at the surface of the native protein and these epitopes are thus poor structural mimics of the antigenic regions of native proteins. It is only because antiprotein antibodies are polyspecific and able to cross-react with short peptides corresponding to separate segments of complex discontinuous epitopes, that large numbers of continuous epitopes have been described.
It is often mistakenly assumed that it is justified to extrapolate from cross-reactive antigenicity (binding of peptides to antiprotein antibodies) to cross-reactive immunogenicity (raising antipeptide antibodies able to cross-react with the cognate protein). However, the structure of an epitope determined when it is complexed with a paratope tends to differ from the structure present before the process of mutual adaptation that occurs when the two binding partners interact. The structure of an epitope after complexation with a neutralizing Mab is therefore an unreliable guide for defining which vaccine immunogens are needed to elicit protective neutralizing antibodies.

Pharmacodynamic Monitoring of Calcineurin Inhibition Therapy

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Introduction: Calcineurin inhibiter (CNI) therapy by cyclosporine or tacrolimus is still considered the backbone of immune suppression after allograft transplantation, especially early after transplantation. Since both CNIs show unpredictable pharmacokinetic profiles, TDM is used to control drug exposure. Unfortunately, CNI therapy is still associated with severe side effects such as nephrotoxicity and unfavorable cardiovascular risk profile. To optimize CNI therapy, more accurate monitoring strategies are required and a pharmacodynamic strategy constitutes a novel and innovative approach.
Content: Several pharmacodynamic monitoring strategies have been developed that all showed inverse relations between CNI concentration and pharmacodynamic markers, such as calcineurin (CN) activity and T cell activation transcripts. These markers have been investigated for allograft recipients treated with CNIs and although the first signs of their usefulness have been reported, they are not suitable for routine monitoring so far. A feature of many pharmacodynamic monitoring strategies is that the large inter-individual variation observed does not necessarily explain clinical and immunological observations. We have investigated whether sample choice and composition could contribute to the observed large inter-individual variation of these markers. We have therefore monitored sample composition when either total leukocyte fraction or peripheral blood mononuclear cells (PBMCs) are chosen as sample in renal allograft recipients. Next cellular CN activities and CN inhibition profiles for cyclosporine were determined. We found that based on the differences in cellular CN activity, the large variation in sample composition could directly confound measurement outcome. Cell-specific CN activity and drug sensitivity should therefore be considered for sample validation.
Conclusions: The call for more accurate monitoring strategies for immunosuppressive therapy has led to the development of a large variety of pharmacodynamic monitoring strategies. The first indications of their clinical relevance are available, but further understanding of the analytical and clinical variables involved seems to be required before these markers can be tested in a clinical setting.

RNA-modified dendritic cells as therapeutic cancer vaccines: Dressed for success ?

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Messenger RNA (mRNA)-based gene transfer has gained an enormous interest over the last decade, especially in the field of immuno-gene therapy of cancer. In this area, most researchers have exploited low voltage electrical pulses (electroporation) as a means to introduce coding RNA into the cells. mRNA electroporation has become a method of choice for transfecting dendritic cells given its superior cytoplasmic expression efficiency, its simplicity over viral transduction protocols and its clinical safety profile because of a strictly transient expression profile and the inability to integrate into the host genome. Furthermore, it allows the simultaneous introduction of antigens and imunostimulatory proteins into dendritic cells through co-electroporation of multiple RNAs. Recently, optimized strategies to produce highly translatable mRNA and more insights into the immunostimulatory properties of RNA structures further advocates the use of RNA for vaccination purposes. Here, I will discuss our own experience with RNA-electroporated DC both in cancer and HIV. I will also present latest data on our recently performed phase I clinical trial using RNA-electroporated DC in leukemia patients. In conclusion, RNA loading by electroporation provides a versatile gene therapy tool for the design of DC-based therapeutic vaccines.

A functional steroid-binding element in an ATP-binding cassette multidrug transporter

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ABSTRACT: The human breast cancer resistance protein is an ATP-binding cassette (ABC) multidrug transporter that affects the bioavailability of chemotherapeutic drugs, and can confer drug resistance on cancer cells. It is the second member of the ABCG subfamily, other members of which are associated with human steroid disorders such as hypercholesterolemia, sitosterolemia, and atherosclerosis. The molecular bases of protein-steroid interactions in ABC transporters are unknown. Here, we identify a steroid-binding element in the membrane domain of ABCG2 with a similarity to steroid hormone/nuclear receptors. The element facilitates steroid hormone binding, and mediates modulation of ABCG2 activity. The identification of this element might provide an opportunity for the development of new therapeutic ligands for ABCG2.

The natural steroidal withanolide Withaferin A is a novel promising chemosensitising compound in B-cell chronic lymphocytic leukemia and metastatic breast cancer"

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Background: B-chronic lymphocytic leukemia (B-CLL), the most prevalent leukemia in adults in Western countries, still remains incurable. In aggressive metastatic breast cancer cells, loss of hormone receptors frequently leads to hormone therapy resistance.
Aims: We wanted to investigate whether Withaferin A (WA), a natural steroidal withanolide purified from Withania Somnifera (Ashwagandha), is able to induce apoptosis in B-CLL and hormone refractory metastatic breast cancer cells and we explored the molecular mechanisms involved.
Methods: We determined IC50 values of WA in B-CLL samples and metastatic breast cancer cells and evaluated various markers of apoptosis. Furthermore, we studied effects of WA on NF□B/Stat3 signaling pathways involved in B-CLL and breast cancer survival.
Results: WA induced apoptosis in B-CLL and metastatic breast cancer cells with IC50-values of 0.60±0.28 and 50±25 µg/ml respectively. Cell death was associated with impaired NF□B activation and increased protein ubiquitination levels, independently of 20S proteasomal inhibition. This correlated with selective downregulation of various B-cell growth factors and anti-apoptotic gene products, including IL-6, IL-8, XIAP, A1/bfl1, Bcl-2. In metastatic breast cancer cells, we found that anti-invasive activity of WA results from cumulative inhibition of constitutive and inducible regulatory steps in the canonical and noncanonical NF□B-signalling pathways. This allows WA to potently repress expression of interleukin (IL6, IL8) and metalloprotease (MMP3, MMP9) survival factors.
Conclusions: These data illustrate the potential of WA as a multifaceted NFκB/Stat3 inhibitor and novel promising chemosensitizing compound in B-CLL- and breast cancer therapy.

The Role of the Dopamine Transporter Gene in Smoking and Other Addictive Behaviors

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Background: Genetic analysis of the human dopamine transporter (HDAT or SLC6A3) has been a central research focus since the cloning of the gene and identification of sequence variants in 1992. The most frequently assessed site is a Variable Number of Tandem Repeats (VNTR) present in the 3'-untranslated region of the gene. Assessment of smoking and addiction-related traits that have a genetic component is made difficult due to the complex nature of these phenotypes. A scan of the literature suggests that new approaches to genetic analysis of smoking, which is the focus of this presentation are needed.
Methods: We analyzed gene-by-environment (G X E) interactions in an intervention program. Children selected at 5 or 6 years old based on ratings of difficult behavior in the classroom were given interventions including training for individual behaviors, interactions with peers, and parent-child interactions. A control group from the same schools, but without specific interventions, was used for comparison. Assessment of smoking and alcohol use in school grades 9, 10, and 11 was used as the outcome variables. There were 1199 participants of which 695 were successfully genotyped, resulting in a group that was 63% male, 48% European-American, 48.6% African-American for this study. The VNTR genotype was assigned as either 9-copy present or absent. Logistic regression was used to assess the effect of genotype by intervention status on frequency of smoking and alcohol use.
Results: The present G X E study showed significant effects of the combination of genotype and intervention on drug use, while little of significance was seen for the main effect of HDAT. Those individuals that possess the 9-copy allele and given the intervention had lower levels of smoking and alcohol use.
Conclusions: Published results have been mixed as to any consensus for a role for variants of HDAT in smoking and other addictive behaviors. Our G X E study showed significant effects suggesting that interactions might have larger consequenses than main effects of the genes themselves.

New perspectives in the treatment of melanoma by a glycobiological approach: results in mouse experimental model

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Background: Glycosylation products contribute to the structure and function of proteins and lipids on the cell surface (cell-cell interactions and immunogenicity). Lectin-like receptors expressed on natural immunity cells (especially NK cells), can recognize transformed cells by their aberrant glycosylation products, as well as tumor microenvironment (TME) degradation products of carbohydrate structures. Multivalent glyconjugates terminated with GlcNAc (GN) and high affinity for NKR-P1 receptor were synthesized. Aim: To test biological and immunological effects of GD in mouse melanoma model.
Methods: Melanoma B16F10 in vivo (C57BL/6 mice) and in vitro;GN: PAMAM-GlcNAc8, PAMAM-GlcNAc4; evaluation of tumors growth, animal survival; FACS analysis and cell sorting of immune cell subpopulations; cytotoxicity; ELISA; western blotting; light, fluorescence, and confocal microscopy.
Results: GN modulated both immunity and cancer cells: 1) B16F10 cells changed their glycophenotype and immunogenicity; 2) immune cells generated a Th1 response (increased IL-2, IFN-γ); 3) cancer growth was slowed; 3) animal survival increased (33% than control). GN administration after temporary inhibition of the disregulated TME immunity, triggered specific-target antitumor response. CD69 activation marker expression and IFN-γ production accompanied recovery of cytotoxic cell activity. In vitro tests showed functional interactions between GN, NK1.1 and NKG2D receptors. GN are internalized by both immune and melanoma cells with different cellular localization and effect (NK cells showed colocalization of the GN with the NK1.1 receptor). Recently, either new GlcNAc-terminated multivalent molecules, or Pleurotus ostreatus mushroom derivatives (containing glucans and chitin) were also found to modulate the melanoma growth and immunity.
Conclusions: Data indicate: 1) the possibility of tumor-immunity modulation by GlcNAc-rich multivalent molecules inside the cancer microenvironment; 2) perspective suitability for immunotherapeutic interventions. Acknowledgements: grants IAA 500200509, IAA 500200510, IAA 500200620 and 310/08/H077;IRC AV0Z50200510(CZ); ARPA Foundation, Pisa (IT). E-mail: vannucci@biomed.cas.cz
Concize statement on the research: Multivalent glycoconjugates targeting NK activation lectin-like receptors modulates mouse anticancer immunity in vivo

Preliminary results of the Semmelweis Budapest Mastocytosis Center

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Background: The Semmelweis Budapest Mastocytosis Center has been started to be organized from the end of 2006, in Semmelweis University of Medicine. There was a wish to join European Competence Network on Mastocytosis (ECNM). Mastocytosis is a rare group of disorders has not been registered so far in Hungary. There were anecdotal case reports only in the literature. Joining ECNM the first task was to set up our network at Semmelweis University.
Methods: Patients with characteristic skin manifestations were directed for hematological workup or patients who had bone marrow analysis for other reasons- and mast cell disease was found had also been registered. Laboratory methods in use are as follows: flow cytometry analysis; immuno- histochemistry (CD2, CD5, CD117) and DNA sequence analysis of cKIT gene on exon 17 to determine c-kit mutation, "nested" PCR for FIP1L1-PDGFR alpha mutation analysis. A longitudinal patient follow up with complement panel detection [CH50/ml, concentration of C1q, C3,C4, C1 INH, C1 INH activity, anti-C1 INH (IGG, IGA, IGM) and anti- C1q (IgG) determination] had been introduced as well in the wish to find correlation- if there are any- to general symptoms or to the therapy had been applied.
Results: 27 patients had been registered so far (10 M and 17 F): 8 cases of Urticaria Pigmentosa, Mastocyte Activation Syndrome: 2, Systemic Mastocytosis localized to Bone Marrow (SM-BMM): 7, SM-associated to hematological non mast cell disease (AHNMD): 10.
Conclusions: 1. Authors consider setting up the network itself as significant result. In the next years by raising the number of registered patients - by widening the network to the whole country- and/or by establishing European collaborations - we might found the basis of scientific work as well. 2. This year in November 7-8 our center received the possibility to organize the annual meeting of ECNM.

Cotrimoxazole, an old "magic bullet" finds a new target and benefit in advanced fibrotic lung disease

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Background: Advanced fibrotic lung disease has a prevalence of 175 cases per 100 000 population, but no effective treatment. Despite costly studies across the US & Europe, expensive immune modulating drugs demonstrated no benefit.
The observation of a dramatic improvement in an individual given co-trimoxazole, was reproduced in a further 14 end-stage patients and led to a pilot study.
Method: A double-blind randomised placebo-controlled study of 20 patients treated with oral cotrimoxazole for 3 months.
Results: Clinical & statistically significant improvements in forced vital capacity +21% and walking distance +355 meters (shuttle walking test) at 3 months (p=0.002) compared with placebo. Oxygen desaturation on walking was reduced (p=0.003). MRC 5 point dypsnoea score improved significantly (p=0.05) at 3 months. The SGHRQ showed a reduction in symptoms scores (p=0.05) at 12months. Vascular endothelial growth factor showed a 50% reduction in the active group, and peripheral blood markers of oxidative stress (monocytes ,MCV & GGT)were significantly reduced p=0.0001. Repeat C.T. chest scans after 12 months of co-trimoxazole treatment showed reductions in ground glass change (p= 0.05),suggesting effects in the alveolar region of the lung. On going monitoring of all patients demonstrated equal responses in the placebo group with stability of lung function in all patients at 12 months.
Conclusion: This drug is the first to fulfil the ATS/ERS 2000 criteria of a beneficial response to treatment. The time course of benefit (2-4weeks) is consistent with its effects as an antibiotic with relapse upon cessation & subsequent resistance. We are now investigating possible immune defects, to assess whether an infective agent could initiated the disease.

A new "quasi-adaptive" response to alkylating agents in E. coli cells due to posttranslational modification in S- nitrosylated Ada protein. Igor

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Background: An experimental verification of the original hypothesis - a "quasi-adaptive response (quasi-Ada) to alkylating agent in E. coli. Assessment perspectives of a new phenomenon application in medicine since structure and functional relationship of E. coli Ada-genes and their mammalian counterparts. Aims: 1)To study if S-nitrosylation (instead of S-alkylation in "true Ada") of the key targets in the Ada protein might serve the signal for the quasi-Ada activation and contribute to the adapted cell resistance to challenge nitrosomethylure (NMU). 2)To answer the question if NO donor structure will affect the level quasi-Ada induction 3)To establish the structure of NO signal molecule. 4)To develop optimized protocol for NO donor application as pseudo substrate in experimental chemotherapy.
Methods: A collection of E. coli mutants bearing (alkA::lacZ; alkB::lacZ and aidB::lacZ) operon fusion [Volkert, 1998] was used. Dinitrosyl iron complex ($DNIC_{glu}$) [Vanin, 1999] and a new stable tetranitrosyl iron complex with thiosulfate ($TNIC_{thio}$) [Sanina, Aldoshin, 2000] were studied as NO donating agents. EPR-resonance spectroscopy was applied to detect a formation inside the cells the typical anisotropic EPR signal with g-factor 2,03. To measure the concentration of NO molecules generated we used an ami-NO-700 sensory electrode from in NO Nitric Oxide Measuring System.
Results: Quasi-Ada induction by low toxic $DNIC_{glu}$ could be quantified by 3-5 fold increasing in the levels of alkA and alkB expression and 1,5-2,5 fold decreasing in the rate of mutations and lethal lesions, induced by NMU. $TNIC_{thio}$ was selectively effective in the alkA gene activation and in inhibition of the alkB and aidB genes expression.
Intracellular iron was indispensable for NO-signaling: o-phenanthroline (OP) already prevented the phenomenon. Treatment of the cells with NO donors led to formation the EPR signal with g 2,03, which disappeared after OP cell pretreatment. It appeared that namely $[Fe^{+}(NO^{+})_2]$ - mediated signaling cascade extends to the level of the Ada-gene transcription. In NMU responsive human tumour models of different genesis $TNIC_{thio}$ increased their NMU sensitivity up to 36-92%. The promising results were obtained with some new NO-donors as the "independent" drugs, as well.
Conclusions: The new quasi-Ada phenomenon extends NO functions in genetic signal transduction within the Ada response system. E. coli can be used as a valid model in identification of the new promising regulators of adaptive processes in mammals.
This phenomenon is currently under study.
This work was supported by Russian Foundation for Basic Research (08-04-00228).

Effect of caffeine on quinidine transport to the central nervous system in rats

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Background: Aim of research was to study the effect of caffeine on quinidine transition through the blood-brain barrier (BBB).
Methods: Study was done on Wistar rats. Control group received sc physiological solution 30 min before quinidine administration; test group received sc caffeine 25 mg/kg bw. Quinidine 25 mg/kg bw was administrated into the right a. axillaris. Blood samples were taken from v. jugularis 30, 60, 90, 120, and 240 s after quinidine. After blood sampling, CNS was rinsed with 5 ml of distilled water injected to the left heart ventricle. Rats were sacrificed in same time intervals as for blood sampling. Brain tissue was divided into the brainstem, cerebellum, cerebral hemispheres. Quinidine concentrations were determined by standard spectrophotofluorimetric method.
Results: At all time points caffeine decreased quinidine transport to brainstem compared to control (except in the 60th s, significant in 90th s). Decrease in quinidine transport to cerebellum was observed in all time points, except in 240th s (statistically significant in 60th, 90th and 120th s). In test group decrease in quinidine transition to left cerebral hemisphere compared to control, was observed in all time points except in 60th s (statistically significant in 90th s). Similar was observed for the right cerebral hemisphere but without statistical significance.
Conslusions: 1) Observed decrease in quinidine transition to brainstem and cerebellum (1st CNS compartment) in presence of caffeine may be explained by effect of caffeine (also a cation) on quinidine transport through the BBB. 2) The hindered passage of quinidine to 2nd CNS compartment (cerebral hemispheres) under the influence of caffeine was less pronounced than in 1st compartment. This may be explained by local conditions of blood circulation, and regional properties of the BBB. 3) The quinidine concentration was higher in brain than in blood. Increased level in brain was registered with a latency compared to blood levels what could be explained by the involvement of active transport of quinidine, as well as by the fact that at higher physiological pH values, quinidine is mainly present in undissociated form. 4) Regional differences observed in quinidine transition to the brain indicate that there are some local factors influencing active transport.

The Prognostic Value of Genetic and Phenotypic Markers of Drug Metabolism and Host and Exposure Factors for Antituberculous Drug Induced Hepatotoxicity

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Background: The intensive antituberculous treatment regimens have as a concomitant result the high frequency of adverse reactions including hepatotoxicity. Estimation of patient individual genetic features in antituberculous drugs metabolism assume as a perspective approach to decreasing risk of these reactions. However discordance of genotype and phenotype in drug metabolism is possible.
Methods: We investigated thepossibilityof the hepatotoxicity prediction in patients receiving isoniazid, rifampicin, pyrazinamide and ethambutol on the basis of isoniazid pharmacokinetics and genetic polymorphism of arylamine N-acetyltransferase 2 estimation. It was detected 282C>T, 481C>T, 590 G>A, 803G>A and 857G>A SNP's in 75 patients with lung tuberculosis and in 52 patients was detected pharmacokinetics of isoniazid in first days of therapy.
Results: Distribution of pharmacokinetic parameters of INH elimination (constant of elimination, Kel, clearance total (Cl) and time of half-elimination (t1/2) was bimodal with antimodes 0,2 h^{-1}; 3,5 ml/min/kg and 3,2 h, respectively. Median values of t1/2 were equal 6,44 h for slow and 1,8 h for rapid acetylators. Frequencies of 481T, 590A and 857A alleles were 0,36, 0,29 and 0,06, respectively. In group of examined patients were 13 cases (26%) of inconsistency between genetically predicted and pharmacokinetically determined acetylation phenotype, 11 of them were dislocation of genetically fast acetylators into phenotypically slow acetylators. Level of alanine aminotransferase (ALT) was elewated during first month of therapy in genetically as well as pharmacokinetically determined slow acetylators. However level of statistical significance of differences between initial values of ALT with those after 1, 2 and 3 month of therapy was higher in analysis with pharmacokinetic data.
Conclusions: The pharmacokinetic estimations are more preferable for individual prognosis of drug-induced hepatotoxicity.

Can Cardiovascular Drugs Influence Clinical outcome in CABG patients

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Background: The aim of our study was to analyze the effect of cardiovascular therapy on clinical outcome in Coronary Artery Bypass Graft surgery (CABG) population.
Methods:
A total of 494 CAD patients, treated with CABG surgery were analyzed. Pre and postoperative usage of cardiovascular (CV) therapy: digitalis, diuretics, aldosteron antagonists, ACE/ATRB inhibitors, beta blockers, Ca channel blockers, ASA alone or combined with oral anticoagulants, statins, nitrates and several other drugs were analyzed. Early (hospital morbidity and mortality) and late clinical outcome was followed during the period of 8,5 ± 4,6 y.
Results: ASA alone or combined with oral anticoagulants applied early before CABG was found to be an independent predictor of reduced hospital mortality (OR 0,660; beta -2,818; p=0,000), acute heart and renal failure (AHF/ARF) and infections. In long term follow up, it reduces the risk of heart failure (HF) and myocardial infarction (OR 0,259; beta -1,692; p=0,048). Alone or together with oral anticoagulants, ASA is an independent predictor of reduced CAD progression (OR 0,137; beta -1,987; p=0,000) and need for re-revascularization (OR 0,169; beta -1,780; p=0,000). Postoperative ASA continuation reduces the risk for cardiac death (CD) (OR 0,360; beta -1,022; p=0,082), HF (OR 0,555; beta -0,588; p=0,019), CAD progression and re-revascularization. Surprisingly, statins increased the risk of arrhythmias early postoperativly (OR 2,920; beta 1,072; p=0,005), but they reduced the risk of AHF/ARF and late HF. They were also found to be independent predictors of reduced CAD progression (OR 0,378; beta -0,972; p=0,010), re-revascularization (OR 0,257; beta 1,276; p=0,022) and CD (OR 0,783; beta -1,180; p=0,034). Ca channel blockers reduce the risk of CAD progression and need of re-revascularization. Beta blockers given pre/postoperatively reduce the risk of HF (OR 0,455; beta -0,788; p=0,001 and OR 0,393; beta -0,934; p=0,000), but insignificantly increase the risk of CAD progression and re-revascularization. ACE/ATRB inhibitors gave us the most disappointing results. They were found to increase the risk of ARF/AHF, HF, myocardial infarction, CD, CAD progression and re-revascularization.
Conclusions: 1) ASA and statin therapy should be started as early as possible after the diagnosis of CAD. 2) Ca channel blockers had benefitial effects in the reduction of CAD progression. 3) Beta blockers had benefitial effects in the reduction of HF, but no effect was found regarding to CAD progression. 4) We don't know where is the problem with ACE/ATRB inhibitors in our study?

Physico-Chemical Insights in Biological Conversions; the Role of H_2O as driving Force in Cytochrome P450 catalysis

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Background: For many years the involvement of the oxenoid iron form ($Por^{+}Fe^{IV}$=0) as active component in cytochrome P450 catalysis was generally accepted, despite many open questions.
Method and Conclusions: An alternative approach was made by the study of microperoxidase-8 (MP-8). MP-8 is a peptide of eight amino acids bound by two cysteines to the pyrrole rings of the heme cofactor and ligated to a histidin of the peptide chain. MP-8 acts as a peroxidase in the presence of H_2O_2 that is fully inhibited in the presence of ascorbate, but remains active in different P450-activities. In the peroxidase mode it catalyses rather complicated polymerization processes, with one exception the nitration of phenols. In the P450 mode MP-8 either catalyses electrophilic substitution reactions like hydroxylation reactions or it catalyses nucleophilic substitution reactions like N-dealkylation and in dehalogenation. When the dehalogenation reaction is carried out in either methanol or ethanol, the halogen atom is replaced by the corresponding alkoxy-group.
MP-8 catalyses O-exchange between H_2O_2 and H_2O. Oxygen from water is incorporated in two compounds without any hetero atom, naphtalene and anthracene. This observation indicates that 55.5 M water is the driving force in MP-8 and most probably also in P450 catalysis. Insight into the actual involvement of different heme-species was obtained by electronic structure calculations in local spin-density approximation along the reaction pathway of the model compound hydroxylating its substrate. Only under specific geometry the peroxo-iron species ($PorFe^{III}$-OO^{-}) passes in the hydroxylating reaction pathway with the substrate a maximum around 190-200 pm followed by a minimum at approximately 130-140 pm, the length of an aromatic C-O bond, as well as region-selective substitution..
In Summary: Peroxo-iron can act as the single species in both nucleophilic and electrophilic P450-type of substitution reactions; Since MP-8 has limited stability under turnover conditions design of small and stable heme-iron catalysts is necessary for applying in clean chemical reactions.

Unprecedented Antitumor Effect of Irradiation Generated by 5-Trimethylsilyl-2-Trifluoroacetylfuran Oxime in Fibrosarcoma Cells

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Background: Numerous experiments evidence that spontaneous or artificially induced signalling photon emission (SPE) produced by unicelllular and multicellular organisms serves not only for the regulation of metabolism but also for their biocommunication with surrounding biological systems. Emission, trapping, amplification and other aspects of SPE are mainly confined in the boundaries of appropriate biological system. However its small part break loose in outer space and could be detected both qualitatively and quantitatively using appropriate physical equipment or detectors of biological origin. During antitumor screening of organic substances *in vitro* using monolayer cells, we unexpectedly found that SPE with strong cytotoxic effect could be induced by 5-trimethylsilyl-2-trifluoroacetylfuran oxime (IOS-8596).
Methods: The solution of IOS-8596 was added only in 3 rows of conventional polystyrene 96-multiwell plate containing monolayer human fibrosarcoma cells (HT-1080). Thus prepared plate was cultivated at 37°C for 72 hours. A quantitative assay for the cell population treated and untreated with IOS-8596 was performed by staining with crystal violet (CV) or Coomassie Brilliant Blue R-250 (CB).
Results: It was found, that IOS-8596 killed the cells, not only in the well-emitters in which it was added, but also in adjacent well-detectors containing cell culture alone. The intensity of cytotoxic effect observed in well-detectors directly depended on the amount of IOS-8596 added in well-emitters. The addition of □-carotene in well-detectors at a concentration of 10 □g/ml resulted in the twofold reduction in cytotoxic effect in well-detectors proving the participation of singlet molecular oxygen in metabolic processes induced by SPE. It was found that the intensity of SPE could be effectively regulated by optical fibres by the putting their one end in the well-emitter or in well-detector like antenna. For example the placement of one end of optical fibre with 400-2400 nm diapason of wavelength transmission in well-emitter resulted in 2-fold increase of cytotoxic effect in well-detectors compared with the control.
Conclusions: Thus obtained experimental data evidence about the development of a simple and reproducible method of SPE generation, its detection and investigation in 96-multiwell plate the same as its amplification using optical fibers providing good perspective for non-invasive treatment of cancer.

Dopaminergic Receptors: Potential Therapeutic Applications

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Background: Dopamine (DA) induces a variety of physiological responses in the cardiovascular (CV) and renal systems, which result from its interaction with DA receptors. DA and its agonists have a role in the treatment of high blood pressure (BP), other CV and renal dysfunction.
Methods/Results/Discussion: The neurotransmitter DA, the precursor of noradrenaline, induces a variety of CV and renal physiological responses, including an increase in myocardial contractility and cardiac output without changes in heart rate, passive and active vasodilatation, diuresis and natriuresis. These responses result from its interaction with the DA receptors D1, D2, D3, D4 and D5, and recently discovered D6 and D7 receptors. The expression and functions of the DA receptors are tissue specific. The interaction of DA and dopaminergic compounds with the DA receptors involves cyclic AMP, intracellular calcium, potassium channels, Na+/H+ exchanger, Na+/H+/ATPase pump, etc. The CV, renal and hormonal actions of DA and dopaminergic compounds are dose-dependent. In some types of hypertension. DA is known to influence the control of arterial BP by influencing the central and peripheral nervous system and target organs such as the kidneys and adrenal glands. DA and DA-agonists, including inhaled DA have a role in the treatment of high BP, other CV diseases and renal dysfunction. Hence, it is important to review the physiological and pharmacological aspects of DA and its receptors, and the potential clinical utility of DA and its derivatives in the therapy of hypertension, other CV diseases, and renal dysfunction.
Conclusions: DA is an important neurotransmitter with varied physiological action in the CV and renal systems. DA and DA-agonists, including inhaled DA have a role in the treatment of high BP, other CV diseases and renal dysfunction.

Preoperative chemoradiation with concurrent Capecitabine for Locally Advanced Resectable Rectal Cance (LARC): a Phase II study

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Background: A prospective one-arm phase II study was performed between 6/2004-1/2005 to assess efficacy and toxicity of preoperative radiotherapy (RT) and concurrent oral capecitabine in patients with LARC.
Methods: Patients were irradiated to 45 Gy in 25 fractions over 5 weeks to the pelvis concomitantly with oral capecitabine 825 mg/m2 bid including weekends. Surgery was scheduled 4-6 weeks after completion of the chemoradiotherapy. Four courses of chemotherapy were planned postoperatively. The primary endpoint of the study was complete response rate; secondary endpoints included toxicity, survival and long-term rectal and urogenital morbidity. Patients still alive and without recurrence of the disease, with a minimum follow up of 1 year, were questioned with LENT/SOMA late-effect scale for rectum, bladder and sexual function.
Results: Fifty-seven patients entered the study (median age 67 years, 43 males and 14 females). During preoperative part, one female died after receiving 27 Gy (pulmonary embolism) and the most frequent grade 3 toxicity was dermatitis (33.9% of patients). Radical operation was performed in 55/57 of patients. The complete pathological response rate was 9.1%. T-, N- and overall downstaging rates were 40%, 52.9% and 49.1%, respectively. A total sphincter preservation rate was 65.5%. During the early perioperative period, one patient died due to sepsis. Five patients (10%) were reoperated due to anastomotic leakage, intraabdominal abscess, ileus, enterocutaneous fistula and stomal occlusion. At 1 year of follow-up, the rate of patients with severe late (SOMA grade 3 and 4) rectal, bladder and sexual toxicity was 40%, 19.2% and 31%, respectively. The local relapse has occured in 1 (1.8%) patient and disemination in 13 (24%) out of 54 patients with median time to progression 23 months (range 3-23 months). Second primary malignancy has occured in 2 patients. The actuarial 2-year (median follow-up) OS, DFS and DSS rates were 84.2%, 72.5% and 92.4%, respectively, and local control was 98.2%.
Conclusions: Preoperative chemoradiotherapy with oral capecitabine is well tolerated, safe and convenient treatment regiment of LARC. It results in excellent local control. The rate of distant relapse and late functional morbidity is still of concern and asks for new treatment approaches

Vulvovaginal Colonization by Aspergillus Species in Nonimmunocompromised Women

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This study was undertaken to determine the prevalence of female genital Aspergillus infections. Additionally, the study explored whether genital Aspergillus infections were associated with Aspergillus infections elsewhere (e.g., pulmonary, cardiac, orthopedic, or ophthalmologic). Between October 2005 and October 2007 vulvovaginal fungal cultures were performed for all patients seen in the Vulvar Disorders Center at the Good Samaritan Hospital (Cincinnati, OH) and similarly at Wright State University Boonshoft School of medicine Department of Obstetrics and Gynecology (Dayton, OH). Prospectively, any cultures showing Aspergillus species were segregated and a running list totaling 16 patients was maintained. The prevalence for Aspergillus in the vaginal culture was 6 per 1,000. The patients were all followed, examined and cultured at each subsequent visit for genital and extragenital disease. The patients responded to therapy with a culture-proven elimination of the fungus. Sporanox® (itraconozole; Janssen Pharmaceuticals, Titusville, NJ) 200 mg daily for 30 days proved to be the most objectively effective agent for fungus elimination. No systemic Aspergillus infections were observed during the follow-up period.
Disclosure: The Authors have no connection to any companies or products mentioned in this study.

Hyaluronan Mixed Esters of Butyric and Retinoic Acids: Multicomponent-Multitarget Drugs with Differentiating and Paracrine Logics for Cardiovascular Repair with Human Mesenchymal Stem Cells

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Cell lineage specification is fashioned at multiple interconnected levels and is controlled by a complex interplay between cell signaling, nucleosomal assembly, the establishment of multifaceted transcriptional motifs and the temporal and spatial organization of chromatin in loops and domains. Stem cells are pluripotent elements that possess the unique capability to self-renew, grow indefinitely and develop into multiple types of cells and tissues and may both offer a clue for the identification of specific differentiation patterning and hold a promise for rescuing damaged tissues. The demonstration of a multineage potential in human mesenchymal stem cells has prompted such a perspective even in humans. Within this context, the development of strategies affording high-throughput of targeted commitment from pluripotent cells would have obvious therapeutic potential. However, overexpression of genes coaxing stem cells to a specific lineage by vector-mediated gene transfer is a cumbersome approach that may perturb normal homeostasis in both stem cells and recipient tissues, and is not readily envisionable in humans. It is now evident that the identification and/or the development of molecules which induce selected commitment in pluripotent cells would help to elucidate the molecular mechanisms underlying complex differentiating processes and may ultimately allow us to regenerate tissues in vivo. Recent developments in the area of stem cell research have been boosted by an increasing understanding of transcriptional regulation and epigenetic modifications, including histone acetylation, DNA methylation and chromatin remodeling. Within this context, we have developed hyaluronan mixed esters of butyric and retinoic acids (HBR), eliciting a remarkable increase in the transcription of cardiac lineage-promoting genes, and cardiac differentiation in mouse embryonic stem (mES) cells, ensuing in a high yield of spontaneously beating mES-derived cardiomyocytes [1]. On the whole, these results demonstrate the potential for chemically modifying the gene program of cardiac differentiation in stem cells without the aid of gene transfer technologies and may pave the way for novel approaches in tissue engineering and myocardial regeneration [1]. Recently, we have used HBR to successfully coax human mesenchymal stem cells from bone marrow (BMhMSCs) and alternative sources, including the dental pulp (DPhMSCs) and fetal membranes of term placenta (FMhMSCs), towards a cardiovascular decision in vitro [2]. In each cell population, HBR increased the transcription of cardiogenic and vasculogenic genes, leading to the appearance of cardiac marker-expressing cells and endothelial cells. Interestingly, HBR primed a remarkable and sustained increase in the secretion of both "Vascular Endothelial Growth Factor (VEGF" and "Hepatocyte Growth Factor (HGF)", acting in a paracrine fashion as trophic mediators that not only possess angiogenic but also cardioprotective effects, including antiapoptotic, mitogenic and antifibrotic activities [3,4]. Under our experimental conditions, the differentiating and paracrine effects primed by HBR in vitro were considerably more pronounced in FMhMSCs than in BMhMSCs or DPhMSCs. Transplantation of FMhMSCs preconditioned ex vivo with HBR into the hearts of rats subjected to acute myocardial infarction by coronary ligation led to complete normalization of myocardial performance and dramatic reduction in scar formation [2]. The injection of HBR-treated cells was followed by a significant increase in density of capillaries negative for anti-human von-Willebrand Factor (vWF) (due to vasculogenic responses from the recipient tissue), indicating that enhanced in vivo release of VEGF and HGF by HBR-pretreated FMhMSCs may have contributed to the observed cardiac repair. Besides this, in the hearts injected with HBR-exposed FMhMSCs, the yield of cells positively stained with a human-specific anti-vWF antibody remarkably exceeded the number of vWF-positive cells detected in samples from the untreated group [2]. A consistent organization of human vWF positive cells into erythrocyte containing capillary vessels was only observed in hearts transplanted with HBR-treated FMhMSCs. Hence, the vascular lineage commitment primed by HBR in vitro was retained within the transplanted cells in the infarcted myocardium, suggesting that HBR-treated cells may also contribute to neovascularization and heart rescue through their ability to generate capillary-like structures. Some of the transplanted cells that had been pretreated with HBR also expressed cardiac-specific markers, although the physiological properties of this cardiac-like cells and their contribution to cardiac rescue remain to be assessed.Noteworthy, xenogeneic FMhMSC transplantation was devoid of immune rejection and did not require immunosuppressant procedures. This observation and the finding that FMhMSCs exhibited larger differentiating and paracrine responses to HBR compared to DPhMSCs or BMhMSCs may be relevant for future development of new chemistry designed for targeting stem cell fate and therapy. In fact, recent evidence suggests that age and disease states affect the collection of sufficient healthy autologous bone marrow-derived stem cells, which will decrease the ability of autologous cells to rescue infarcted hearts [5]. FMhMSCs do not induce allogeneic or xenogeneic lymphocyte proliferation and actively suppress lymphocyte responsiveness [6]. Moreover, FMhMSC transplantation in neonatal swine and rats resulted in human microchimerism in various organs and tissues [6].In conclusion, novel chemical agents with differentiating and paracrine logics may be used in combination with tolerogenic human mesenchymal stem cells to afford unprecedented strategies of stem cell therapy and regenerative medicine.

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Newer antifungal drugs in kidney transplant recipients

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Background: Infection is a common cause of morbidity and the second cause of death after cardiovascular disease in renal transplant recipients . Although renal transplant recipients have been thought to be at low risk for fungal infections compared with other transplant recipients, the rate of hospitalization for fungal infections is much higher than the general population. Aspergillosis in renal transplant patients is the commonest cause of systemic fungal disease with an incidence ranging from 0.4 % to 2.4 % with a high mortality of 56-100%. Fluconazole is the treatment of choice for fungal infections; however invasive candidiasis and aspergillosis are relatively less susceptible to fluconazole. For many years, amphotericin B has served as the mainstay for the treatment of invasive fungal infections. However, infusion related toxicity, nephrotoxicity and electrolyte disturbances have limited its use; moreover, the efficacy of amphotericin B is relatively limited with a mortality rate in treated patient that exceed 80%. Newer antifungal drugs, such as caposfungin and voriconazole, have been introduced recently in the treatment of invasive fungal infections and aspergillosis: these drugs have demonstrated to be as effective as amphotericin B in the treatment of invasive fungal infections with less drug-related side effects.
Methods: From January 2002 to December 2007, 247 kidney transplants have been performed at Organ Transplant Unit of University Hospital of Catania. Among these, 17 recipients developed an invasive fungal infection (13 an oesophageal infection and 4 an urinary tract infection), while 4 patients developed an invasive aspergillosis. The patients with invasive fungal infection have been treated with caspofungin, administered once-daily at a loading dose of 70 mg followed by 50 mg/die for a median time of 12 days. We observed a complete relief of symptoms in all patients. Caspofungin was well tolerated, with no signs of drug related nephrotoxicity or hepatotoxicity. Four patients with invasive aspergillosis were treated with voriconazole, starting with a dose of 200 mg b.i.d. and was administered for a period of 60 days. All patients experienced a complete relief of symptoms, without recurrence of aspergillosis.
Conclusion: Newer antifungal drugs, such as voriconazole and caspofungin are potent and well-tolerated antifungal therapies that are extremely efficacious in the treatment of invasive aspergillosis and invasive fungal infections in kidney transplant recipients. A careful monitoring of immunosuppressive drugs should be considered to avoid nephrotoxicity.

Cardiac Hormones: Magic Bullets for the Treatment of Congestive Heart Failure, Renal Failure and Cancer

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Background: William Harvey in 1628 discovered that the heart was a pump. It was another 350 years before it was discovered that the heart was a sophisticated endocrine organ making a family of peptide hormones. One gene in the heart i.e. the atrial natriuretic peptide gene synthesizes four peptide hormones, i.e. long acting natriuretic peptide (LANP), vessel dilator, kaliuretic peptide and atrial natriuretic peptide (ANP). These hormones are important in controlling blood pressure and maintaining blood volume by enhancing excretion of sodium and water.
Methods: These investigations of cardiac hormones involved in vitro study of cancer cells for their mechanism of action and in vivo studies of human cancers in athymic mice (n=30, for each cancer) where the cardiac hormones were infused for 28 days at a concentration of 3 nM/kg body weight/min.
Results: One of these cardiac hormones, i.e. vessel dilator causes a 5-fold excretion of sodium and water while at the same time enhancing cardiac output in persons with congestive heart failure. This same "magic bullet" decreases elevated creatinine (eight average) to normal after six days of acute ischemic renal failure in animals and regenerates the nuclei in the tubules. These peptide hormones eliminate up to 97% ($p<0.001$) of human pancreatic, breast, colon, ovarian, kidney and prostate adenocarcinomas, glioblastomas of the brain, as well as small-cell and squamous cell lung carcinoma cells in vitro. When infused subcutaneously they eliminate up to 80% of the human pancreatic adenocarcinomas. The treated animals lived a normal lifespan. Similarly, these peptide hormones eliminate two-thirds of human breast adenocarcinomas and up to 86% of human small-cell lung carcinomas growing in athymic mice without surgery. These cardiac hormones mechanism(s) of action in cancer cells includes a 97% inhibition of the phosphorylation of extracellular-signal regulated kinases (ERK) 1/2 and of the upstream mitogen-activated protein kinases MEK 1/2 ($p<0.001$) ANOVA. These inhibitions are mediated by the intracellular mediator cyclic GMP.
Conclusion: These "magic bullets" synthesized by the heart are truly "magic" as they have beneficial effects in several diseases that cause a large portion of the morbidity and mortality in mankind, i.e. congestive heart failure, renal failure, and cancer(s).

What happens with the large DNA when it meets intercalating drugs?

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Background: DNA intercalating drugs locally distort a regular DNA conformation. Usually any local changes of helical parameters cause some biological impact e.g. steric hindrance and change of binding affinities for DNA associated enzymes. In case we know the molecular target, we are able to estimate optimal but efficient dosage of drug to eliminate many side effects. DNA is a huge macromolecule containig many potencial places for intercalator binding. The sum of local changes causes a great global change in chromosomal DNA structure. DNA intercalation might be a reason for chromosome rearagement and reassembling, causing inappropriate gene switching on and off.
Methods: Principal method is the temperature-gradient gel electrophoresis. The complemetary methods AFM, UV-VIS spectroscopies and for cell cytotoxicity MTT test have been used. Data obtained on the plasmid system were extrapolated to chromosomal DNA.
Results: The table summarizes results obtained for selected intercalators, the reversibility of intercalator (ability to dissociate from DNA), where C_{ex} represents the concentration of drug measured by TGGE in 50 mM Tris–HCl (pH 7.8) extrapolated to chromosomal DNA and 5mM $MgCl_2$. IC_{50}, concentration required to reduce the number of living cells to 50% as determined by dose–response curves using the MTT assay. The ratio in the last column gives information about the possibility to achieve toxic concentration inside the cell.

Drug	Reversibility	C_{ex} (□M)	IC_{50} (□M)	Ratio C_{ext}/ IC_{50}
Actinomycin D	-	1.0 ± 0.2	0.001 – 2.0	0.5 - 1000
Daunorubicin	+	1.3 ± 1.0	0.02 - 3.0	0.33 - 65
Ellipticine	+	2.5 ± 1.2	0.3 - 4.0	0.3– 8.3
Quinacrine	+	2.7 ± 1.8	1.6 - 2.2	1,2– 1.7
Quinine	+	225 ± 180	~40	~5.5
Quercetin	-	230 ± 150	20 - 50	4.6 – 11
Chloroquine	-	20 ± 22	17 - 33	0.6– 1.2

Conclusions: For drugs where C_{ex} are ranked at 20–230 is not considered to be a standard cytotoxic drug, most likely because the probability that the intercalator will attain cytotoxic concentrations, eliminating any non-B structure from the DNA inside the cell. Therefore, only the cases where r<1.3 and and C_{ex} <10 mM could play a significant biological role in forming alternative non-B structures. When the ratios are lower, cytotoxicity must be mediated by other processes such as the direct inhibition of certain metabolic pathways.
Authors' disclosure statement: If the cytotoxic concentration is lower than Cex, then the effect of intercalator to DNA and on the formation on alternative non-B conformation is not caused by this drug.

Desipramine and Panic: Complex Approaches to Understand Complex Interactions in Psychopharmacology

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Background: Clinical studies have shown that some antidepressants may be more efficient than benzodiazepines to alleviate anxiety associated with panic disorder. Nevertheless, operant and open-field behavioral procedures developed in rats so far do not seem particularly able to model human anxiety sensitive to antidepressant treatments. Researchers do not usually statistically subtract the effect of confounding factors from the variables of interest.
Methods: Undernourished rats, which showed a generalized activation of noradrenergic system, were selected due to their neurobiological resemblance to human patients suffering from panic disorder. The Geller- Seifter paradigm (under a cross over design) and the open field drink test (under an all-inclusive behavioral design) represented, respectively, the learned and ethological conflictive conditions in adult life. Desipramine (10 mg/kg/day) or saline were administered IP during 7 days to approximately ten rats per experimental group. Both studies were contemplated under a multifactor and multivaried perspective.
Results: In the Geller-Seifter paradigm, the repeated measure Anova with Diet as the independent variable and Drug performances (Saline and Desipramine) as the dependent ones indicated non-significant effects of Diet or Drug. However, a significant Diet x Drug interaction was observed in the complex dependent variable, which represented the level of "suppression/ suppression release". I.e., deprived rats showed a statistically demonstrated suppression release compared to control rats in the complex operant performance. The Diet x Drug interaction was independent of the effects of treatments on milk consumption, reactivity to the electric foot shock, unpunished responding, weight and decision-making. In the Open Field Drink Test, the four dependent variables selected by factor analysis indicated also a significant Diet x Drug interaction in the two- way Manova. This interaction was independent of the effects of treatments on weight or intake and was expressed, on deprived rats, as a decrease in all the selected open field-behaviors except for the time of drinking with respect to the control rats, which displayed, in general, a decrease in all the behaviors except for the frequency of grooming.
Conclusion: The Diet x Drug interaction was interpreted as a selective anticonflict effect of desipramine on subjects predisposed to develop panic-like expressions. Complex approaches allow more complete inferences than those which contemplate only one target behavior

Study of Tenoxicam on Various Drug Delivery Systems

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Background: Tenoxicam is an antiinflammatory and analgesic agent which absorbs completely from the G.I. tract by oral route. It undergoes first pass metabolism and causes ulceration. Hence through the buccal and colon specific delivery system the drug circumvents the G.I tract and avoids the first pass metabolism and also the side effects frequently associated with oral administration like ulceration. Aims: (1) To evaluate the natural flax seed polymer by the buccal and colon specific delivery for tenoxicam.(2)To study the effect of the polymer for the bioavailability of the drug.
Method: The drug tenoxicam in the form of tablets containing various concentration of flax seed polymer (20,30 and 40mg) were evaluated using invitro and invivo methods. The mucoadhesion of the tablets were evaluated using porcine buccal mucosa. The tablets had been subjected to invitro drug release studies at a pH 6.8 phosphate buffer. The Invivo study had been performed with 16 healthy human volunteers. In colonic delivery tablets of tenoxicam were prepared by compression coating with 400, 450 and 500 mg flax seed polymer. The formulated tablets were subjected to invitro drug release studies in simulated colonic fluids (4% w/v of rat cecal contents). The Invivo study had been performed in 6 healthy human volunteers.
Results:. The cumulative percentage release of tenoxicam at pH 6.8 phosphate buffer were found to be 98.20±0.08, 91.01±0.09, 84.39±0.72, 78.45±0.08. The bioavailability (AUC_{0-t}) of buccal and oral tablets was found to be 2226±228, 3251±409, 3379±269 and 1732±96. The invitro studies of colon specific tablets at pH 6.8 phosphate buffer containing 4% w/v rat cecal contents showed that the cumulative percentage release after 26 h were 52.16±0.06, 64.10±0.08 and 98.00±0.19. The Invivo studies conducted in six healthy voltmeters revealed that the drug release was initiated only after 5h (ie) transit time of small intestine and the bioavailability of the drug (AUC_{0-t}) was found to be 2014±210, 2890±220 and 2920±215.
Conclusion:(1) Buccal formulation of tenoxicam containing 40 mg flax seed polymer gives high bioavailability and also had significant mucoadhesive property for clinical application. (2)The colon specific formulation containing 400 mg flax seed polymer proved to have potential carrier for drug delivery into the colon for tenoxicam.

Applications of Pharmacometrics to Immunosuppressive Therapy in Transplant Patients: Can we do better?

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There exists an unmet clinical need and widespread research interest to better understand the dose-concentration-response and adverse events relationships of immunosuppressive medications in transplant patients. The transplant community is missing a significant opportunity to optimize outcomes by focusing predominantly on trough concentrations and the area under the curve (AUC) of the blood concentrations as the most important parameters to correlate with empirical evidence of rejection. This presentation will provide a brief overview of transplant pharmacology and a contemporary view of current and potential use of biomarkers as part of a pharmacokinetically guided therapeutic drug management process. Participants will learn how an improved understanding of the use of biomarker information linked to determinations of blood drug concentrations by PK/PD modeling can lead to individualization and optimization of immunosuppressive therapy in transplant patients.
Learning Objectives:
After attending this session, participants will be able to:

1) Appreciate the importance of PK/PD optimization of immunosuppressive therapy
2) Describe examples of the application of population PK/PD models as part of the therapeutic drug monitoring (TDM) process to improve individualized pharmacotherapy.

Anticancer Effects of Statins

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Background: Despite supporting theoretical reasons as well as experimental evidence, there is still clinical controversy whether statins (inhibitors of HMG-CoA reductase) can prevent from cancer diseases. These contradictory views are based on large epidemiological studies and their meta-analyses. Available data, however, have been focused primarily on cardiovascular outcomes; and secondly, the possible anticancer action of statins have been studied as a group effect. Nevertheless, statins represent a heterogenous group of compounds, which differ in their physical-chemical properties, as well as different pharmacokinetic and pharmacodynamic properties.
Methods: Both experimental and clinical data available from Pubmed, as well as own results on the effect of statins on experimental pancreatic cancer, have been used for analysis of the chemopreventive effects of individual statins.
Results: Large differences in antiproliferative effects among statins used for clinical purposes have been described in available literature. In our experimental study on pancreatic cancer the least efficient statins were pravastatin and atorvastatin, whereas rosuvastatin (despite its low lipophilicity) and cerivastatin were the most effective. These data may account for inconclusiveness of cancer prevalence/incidence among statin users in cardiovascular trials. Provided that pravastatin might be the least efficient statin, recent meta-analytic studies might have been confounded by pravastatin trials. This, indeed, is the case for meta-analysis of 7 trials by Hebert et al. [JAMA 1997], where 3 out of 7 studies involved were pravastatin trials. The same is true for a study by Bjerre et al. [Am J Med 2001] (3 of 5 studies involved), CTT Collaborator´s study [Lancet 2005] (5 of 14 studies involved), as well as meta-analysis by Dale et al. [JAMA 2006] (10 of 20 studies). It also should be noted that data are lacking on cancer incidence in subjects treated with rosuvastatin, which has been introduced to the market only recently and which seems to have higher chemopreventive potential.
Conclusion: It is apparent that only further large and well-designed epidemiological studies may determine possible chemopreventive role of individual statins.

Design and Synthesis of Folate Targeted Chemotherapeutics

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Background: Receptor-specific targeting is an attractive concept to control at molecular level the selective delivery of a drug to pathologic cells. One of the many targeting strategies for cell specific drug delivery exploits the folate receptor (FR), a glycosylphosphatidylinositol anchored cell-surface glycoprotein that is over expressed in some epithelial malignancies. The vitamin folic acid (FA) is the ligand that binds FR with high affinity and is transported into the cancer cell via FR-mediated endocytosis. Consequently, attaching biologically active molecules to FA through releasable self-immolative linkers generates a new class of "magic bullet" that utilize FR for targeted delivery.
Tubulysins are members of a new class of natural products isolated from a Myxobacteria species. While possessing exceptionally potent cytotoxicity exceeding epothilones, paclitaxel, and vinblastine, the natural tubulysins alone are not considered suitable for drug development due to their lack of therapeutic window. In contrast, these compounds are ideal war heads for folate-targeted therapy.
Methods: Here we report a simple general approach for producing naturally occurring tubulysins and novel analogs. Thus, treatment of a fermentation mixture of tubulysins with trifluoroacetic acid (TFA) and consecutive addition of the corresponding carboxylic acid produced a single natural tubulysin (A, B, C, G or I) in excellent yield. Next, the efficient synthesis of folate conjugates of tubulysins was designed. In these water soluble "magic bullets" FA and the war head are connected in regioselective manner via a hydrophilic peptide spacer and a reducible disulfide linker.
Results: The key to the novel one-pot procedure for producing single tubulysin is our discovery that an N,O-acetal can be converted in situ in stabilized N-acyliminium ion and subsequent reacted with a nucleophile. The FA-spacer was synthesized using solid phase protocol. The conjugates were prepared applying an original one-pot synthetic approach. Finally, the release of the parent drug from the conjugate was demonstrated.
Conclusions: These studies demonstrate a universal, simple, and easily scalable synthetic approach to natural tubulysins and their FA conjugates. All compounds exhibit potent cytotoxicity towards FR-positive KB cells. The conjugates have demonstrated their selective targeting of FR-expressing cell lines.

Adenosine-Based Therapies for Hearing Loss

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Background: Oxidative stress is the key element in the pathogenesis of many forms of cochlear injury, for example from noise exposure, cytotoxic drugs and aging. Hearing loss from noise exposure (NIHL) is a leading occupational disease, with more than 10% of the population at risk world-wide. This study investigates adenosine-based experimental strategies to stem NIHL. Here we present the first viable pharmacological intervention that can ameliorate noise-induced cochlear injury in the post-exposure period.
Methods: Wistar rats were exposed to narrow-band noise (8-12 kHz, 110 dB SPL, 24 hours) sufficient to induce permanent hearing loss. Gene expression levels of adenosine receptors in the noise-exposed cochlea were studied using quantitiative RT-PCR. Selective adenosine A_1 receptor agonist Adenosine Amine Congener (ADAC) lacking cardiovascular side effects was administered intraperitoneally (100 µg/kg/day) at time intervals after noise exposure. Hearing thresholds were assessed using auditory brainstem responses (ABRs) and the hair cell loss was evaluated by quantitative histology. Free radical damage induced by reactive oxygen and reactive nitrogen species was assessed using 4-hydroxynonenal and nitrotyrosine markers respectively.
Results: Adenosine A_1 receptors were up-regulated during sustained noise exposure suggesting their role in cochlear response to noise stress. The treatment with ADAC after noise exposure led to a substantial recovery of hearing thresholds at all frequencies tested (4-24kHz). Earlier treatment starting at 6 hours after noise exposure provided greater recovery than late treatment starting at 24 hours post-noise. These results were upheld by increased survival of sensory hair cells and reduced nytrotyrosine immunoreactivity in ADAC-treated cochlea. The activation of A_1 adenosine receptors thus ameliorated damage to the sensorineural tissues in the cochlea, leading to the functional recovery of auditory thresholds.
Conclusions: This study strongly suggests that ADAC could be a valuable treatment for noise-induced cochlear injury in instances of both acute exposure to impulse noise and to chronic or intermittent exposures of longer duration. ADAC may also become a drug of choice for other inner ear pathologies based on oxidative stress.

Authors' disclosure statement:
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Then - Macrocirculation and Antibacterial Treatment – and Now: Microcirculation and Antiviral Treatment

VOGEL GE, BACK TH, BLASINI R, BRANDL R, HECKLER R, VON ITZSTEIN M, KOMM CH, MANYCH M, MÖLLER R, SCHÖTTLER M, SCHREIBER M, SCHWAIGER M

In this era of antiviral treatment inflammation is increasingly being suspected again of also playing a role in cancer, arteriosclerosis, multiple sclerosis, diabetes and arthritis (www.griffith.edu.au). As early as 1860 Robert Koch, and later Paul Ehrlich (syphillis and heart disease), saw a connection between inflammation and arteriosclerosis. The discovery of antiviral treatment against influenza (Mark v. Itzstein, 1993) drew attention to the endothelium (endotheliotropism of the virus). There is no infection that does not develop via vessels, endothelium and coagulation (Norbert Heimburger). Endothelial dysfunction and inflammatory processes are important risk factors. Chronic inflammatory metabolic condition affects almost all cells and their microcirculation. The important thing for us in this regard is that the first contact for the acutely ill patient is a family doctor with a new quality (ESWI III, Portugal, 9/2008, Abstract #1325371, #1324914). Thus we were able to treat as outpatients 233 patients of all ages and both sexes with an influenza infection (confirmed by PCR), none of whom had to be admitted to hospital. We also applied this knowledge in the treatment of patients with acute respiratory tract infections caused by other viruses (RSV et al. reported on 1408 patients) and found that 37% of these patients had developed Community Acquired Pneumonia (CAP). The approach in future will be an exact identification of the virus followed by the development of a specific antiviral treatment. Our guiding principle, which we have followed consistently in our approach, namely: „If you understand influenza you understand the whole of virology" (England 1950), summarizes all the knowledge and experience gained with regard to microcirculation and microinflammation that should be applied forcefully. The costs for the health care system in Germany alone are predicted to reach 100 billion Euros – a worthwhile endeavour.

Budesonide - Breath-giving innovation for asthma patients

VOLOVITZ B

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Background: Asthma affects an estimated 300 million people worldwide, causing absenteeism from school and work, disability, and even death. Asthma was initially considered a disease of the smooth airway muscles, and treatment consisted of bronchodilators targeted at reducing the muscle constriction in order to relieve symptoms of wheezing and shortness of breath. It was not until the 1980s that researchers recognized asthma as an inflammatory disease wherein symptoms occur only when the underlying inflammation remains untreated or insufficiently controlled.
Methods: Review of the literature indicates that experts and physicians agreed that maintenance therapy in all patients with persistent asthma should be based on an anti-inflammatory drug, preferably an inhaled corticosteroid. Budesonide is an inhaled corticosteroid introduced in 1981 for the treatment of asthmatic inflammation. It has since been investigated in more than 600 published studies and become the first-line therapeutic agent for asthma. Budesonide is currently approved in 112 countries, and has been used to date on more than 13 billion treatment days.
Results: Budesonide has been shown to be both effective and safe for the treatment of asthma, with improved ratio of topical to systemic potency. Budesonide significantly improved patient health-related quality of life and airway function, and has dramatically changed the life for patients with asthma. Clinical studies found it to be more effective than cromoglycate, theophylline, bronchodilators, and epidemiological studies reported a significant decrease in mortality, hospitalizations, sick-listings, and disability. Budesonide has also been central to many recent evidence-based innovations such as: once-daily administration and protection against both early and late asthmatic reaction and exercise-induced bronchoconstriction. Furthermore, researchers found that with early dose adjustments of budesonide, asthma exacerbations were avoided. Data on the safety of budesonide are extensive and comprehensive. Children treated with long-term budesonide achieved normal final height, and its use during pregnancy and lactation was not associated with adverse effects in either mother or fetus.
Conclusion: Budesonide is effective and safe for the long-term treatment of asthma. Its use has revolutionized asthma therapy and provided patients with a better quality of life.

The Development of Influenza Virus Sialidase Inhibitors as Anti-Influenza Drugs

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The important, and sometimes essential, roles of carbohydrates and the proteins that recognise them in biological processes are diverse [1]. When these roles are associated with a disease a potential drug discovery opportunity is presented. For example, a range of clinically significant pathogens, including viruses, parasites and bacteria utilise carbohydrates and their associated proteins to invade their host, facilitate their lifecycle and as a consequence produce disease [2]. Viruses such as influenza virus, rotavirus, and dengue virus all have essential carbohydrate recognition processes in their replicative cycles that present possible drug discovery targets [2,3].
We have had a long-term interest in influenza virus and the discovery of novel influenza virus sialidase inhibitors that has now provided a novel class of anti-influenza drugs [4-6]. Some of our most recent work and advances towards the development of drugs to treat or prevent influenza, including pandemic influenza, will be presented.
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Chemical Postevolution of Antibacterial Natural Products

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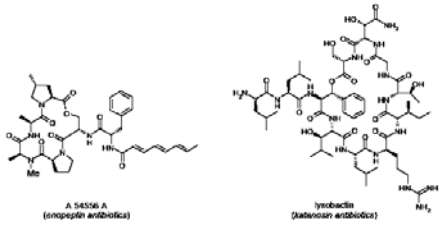
Multi-resistant and hyper-virulent microbes have become a physicians' nightmare in hospitals and in the community (USA300).[1] These aggressive "superbugs" demand "superdrugs" addressing novel therapeutic approaches without cross-resistance to antibiotics in clinical use. Natural products have been the most important source for new antibacterial drug classes.[2] Even today, natural antibiotic lead structures have not lost there value as guideposts for novel targets and future therapy (Reversed Genomics). Most natural products cannot be used in the clinics. However, medicinal chemistry provides the tools to transform natural products into drugs with improved pharmacokinetic and toxicological properties (Chemical Postevolution).[2b]



We have investigated the medicinal chemistry of natural depsipeptide antibiotics such as the katanosines[3] or enopeptins as valuable lead structures on our search for new antibacterial therapies.[4] By their modular structure, cyclic peptide antibiotics[5] are particularly well suited for systematic chemical modifications by means of semisynthesis and de novo synthesis. Synthetic strategies, structure-activity relationships, in vitro potency and in vivo efficacy will be discussed.

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Fentanyl Analogues: Structure-Activity-Relationship (SAR) Study

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Background: Fentanyl is the prototype of the 4-anilidopiperidine class of synthetic opioid analgesics. This study aimed to examine antinociceptive activity of newly synthesized fentanyl analogues supstituted at the position 3, or at the position 4 of the piperidine ring, and to establish structure-activity-relationship (SAR).
Methods: The antinociceptive activity was determined by tail-immersion test in rats of both sexes (200-250 g). All drugs were given intraperitoneally. The antinociceptive activity was reversed by subcutaneous administration of naloxone (non-selective opioid antagonist).
Results: It was revealed that the analgesic activity in both series of 3-supstituted and 4-supstituted fentanyl analogues is influenced by the steric factor (eg. voluminosity of the group and the cis/trans isomerism), and not by the chemical nature of the substituent. The evaluation of an open chain fentanyl analogues, also suggests the influence of the steric factor upon the analgesic activity and in particular, the importance of the piperidine ring as a key pharmacophore.
Conclusions: The potency and the duration of action of these novel fentanyl analogues are interesting from the aspect of SAR studies, and some of them have potential promise for clinical application.

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Wheat and barley for celiac patients by molecular silencing of the immunogenic endosperm proteins

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Celiac disease is the most common food-sensitive enteropathological condition in humans. It is caused by an autoimmune reaction against wheat, barley and rye grain storage proteins. In human leukocyte antigen (HLA) DQ2- (or DQ8-) positive individuals exposure to epitopes of these prolamin proteins leads in celiacs to a painful chronic erasure of the microvilli of the epithelium in the intestine and a permanent intolerance of dietary prolamins. Despite its prevalence in most populations comprising 24.4 million registered celiac individuals world-wide, the only effective therapy is strict dietary abstinence from these food grains. Estimates suggest that for every registered celiac there are 50 unrecognized individuals. Cereal prolamins are of two types: high molecular weight glutenins (HMWG) with a molecular structure of elastic fibrils that form dityrosine cross-links during dough formation and baking, and gliadins. The gene promoters of the gliadin-type proteins, but not those of HMWG, are silenced by DNA methylation in vegetative tissues. This methylation is removed during grain development by a 5-methylcytosine deglycosylase to permit transcription and protein synthesis. Silencing of the enzyme by mutation or by RNAi technology in the endosperm prevents the synthesis of gliadins, which contain the vast majority of the different celiac causing epitopes and only the HMWG prolamins will be produced in fully viable plants. Gliadin-type prolamins are of no importance for baking since wheat HMWG has been demonstrated to be alone sufficient to produce high quality breads. This is probably the first project using interference with DNA de-methylation to obtain a remedy for an autoimmune disease.

Synthesis of a Novel Subunit Vaccine against HIV-1: Native Envelope Proteins in Lipid Bilayer of Inactivated Virions Devoid of p24, RT and Viral Nucleic Acids

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Background: HIV vaccine development remains a continuing challenge. It is our objective to prepare inactivated HIV-1 isolates devoid of p24, RT, and viral RNA, but retaining the quaternary structure of their native envelope proteins. A pool of such isolates derived from human blood can safely elicit protective antibodies against HIV-1.
Methods: Plasma from five blood donors with acute HIV infection (PHIV: NAT+ but antibody-) was used for virus isolation. PHIV isolates co-cultured with PHA-stimulated PBMC from transfusable blood were pooled and reacted with Benzonase (BZ) to hydrolyze free cellular/viral DNA/RNA and filtered through a 300 kD membrane. Beta-cyclodextrin (BCD) was used to extract membrane cholesterol and permeabilize the purified virions, allowing BZ to hydrolyze virion-associated DNA/RNA. The quaternary structure of HIV-envelope proteins (gp41 and gp120) was restored by reloading the virion shells with Cholesterol.
Results: The PHIV isolates co-cultured with single donors' PBMC yielded p24 widely ranging between 2.6-175 ng, whereas PBMC pooled from 3-4 unselected donors uniformly yielded 174-177 ng/million cells. Magnetic beads coated with anti-CD45 removed cellular microvesicles from culture supernatants, yielding purified virions (0.6 ug/ml p24), which contained HIV-RNA ~2X10e9 copies/ml (CpmL). After reaction with 300 or 500 mM BCD, virions were consistently negative in PBMC co-cultures. Results tabulated below show residual p24 and HIV-RNA CpmL after each successive step of viral inactivation.

HIV-1 reacted with	p24 (%)	HIV-RNA
0.3 mM BCD	10	1.1X10e4
0.5 mM BCD	4	6.2X10e3
0.3 mM BCD + 1XBZ	2	2.8X10e3
0.3 mM BCD + 2XBZ	0.8	1.2X10e2
0.5 mM BCD + 2XBZ	0.8	0.8X10e2

Thus, viral inactivation with BCD led to loss of >10e5 HIV-RNA CpmL amplifiable by RT-PCR; this was further reduced to 0.8X10e2 by additional reaction with BZ to hydrolyze virion-associated RNA. Since HIV-1 SF2 vaccine stock contained ~10e5 TCID50 and 6.4X10e9 RNA CpmL, the minimal chimpanzee infectious dose (CID-50) of 1X10e2.3 is equivalent to ~5.4X10e 6.7 CpmL. Thus, combined treatment with 0.3 mM BCD and BZ would provide a product with safety margin that is ~2.6X10e3.7 below the minimal in vitro infectivity in chimpanzees. The forgoing results indicate the safety and efficacy of PHIV inactivation, which eliminated p24 and HIV-RNA but retained ~85-90% of the gp120.
Conclusions: Our preliminary results provide an impetus for pragmatic refinements and a rational basis for seeking FDA-IND and IRB approvals for a pilot clinical trial of the candidate vaccine for immunotherapy in individuals whose HIV infection is controlled with HAART. Ultimately, broadly neutralizing antibody response in uninfected/at-risk individuals would permit serological distinction between infection and protective immunization.

Proteomic analysis of human breast cancer cells derived from metastatic versus non-metastatic tumors

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Background: Breast carcinomas are a heterogeneous group of tumors diverse in behavior, outcome and response to therapy. Identifications of proteins that reflect the tumor biology can improve the diagnosis, prediction, treatment selection and targeting of therapy.
Methods: Primary cultures of epithelial cells from malignant breast tumors were studied by two-dimensional electrophoresis (2DE). Differentially expressed protein spots were identified by mass spectrometry. The samples were obtained from women who underwent partial breast resection or radical mastectomy for breast cancer at the General Faculty Hospital in Prague. The patients were treated according to stage-adjusted therapeutic standards. We estimated the clinical outcome of the patients. The 23 patients with follow-up at least three years were chosen for further analysis. The patients were divided in two groups: distant metastases-free after three years and patients with proven distant metastases. 2-DE gels in pH range 4-7 were prepared. Spot densities in 2-DE protein maps were subjected to statistical analyses and data-mining analysis. Proteins in selected spots were identified using liquid chromatography-tandem mass spectrometry (LC-MS/MS).
Results: Three protein spots were significantly altered between the metastatic and nonmetastatic groups. The correlations were proven at the 0.05 significance level. Nucleophosmin was increased in the group with metastases. The levels of 2,3-trans-enoyl-CoA isomerase and glutathione peroxidase 1 were decreased.

Drug discovery related to vaspin, visceral adipose tissue-derived serine protease inhibitor, a novel adipokine with insulin-sensitizing effects

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Background: To delineate the relationship between increased adiposity and insulin resistance in metabolic syndrome, many researchers have identified adipokines. In such adipokines, TNF-□, resistin and RBP4 (retinol binding protein-4) cause insulin resistance, while leptin and adiponectin enhance the insulin sensitivity. Otsuka Long-Evans Tokushima fatty (OLETF) rat is an animal model of type 2 diabetes, characterized by abdominal obesity, insulin resistance, hypertension, and dyslipidemia. Differential screening of the genes up-regulated in visceral adipose tissues of obese OLETF rats and down-regulated in non-obese and diabetes-resistant Long-Evans Tokushima Otsuka (LETO) rats led to the identification of vaspin (visceral adipose tissue-derived serine protease inhibitor), which is a member of serine protease inhibitor (serpin) gene family. Rat, mouse and human mature vaspins are made up of 392, 394 and 395 amino acids, exhibit ~40% homology with □1-antitrypsin. Vaspin mRNA is exclusively expressed in visceral adipose tissue in genetically obese rats and also expressed in white adipose tissues of human and its expression is correlated with BMI and insulin sensitivity.
Methods: To explore the functional role of vaspin in metabolic syndrome, we prepared recombinant vaspin protein and generated vaspin transgenic (Tg) mice under a control of aP2 promoter.
Results: Under high fat-high sucrose (HF) diet, recombinant vaspin-treated DIO and db/db mice, and vaspin Tg mice revealed improved insulin sensitivity. The body weight of Tg mice was ~8% less than wild type (WT) mice under HF diet. And serum leptin levels were significantly lower in Tg mice (2.7±2.9 ng/ml) compared with WT mice (10.9±6.4 ng/ml). Triglyceride accumulation in the liver is diminished in Tg mice rather than in WT mice. There were no differences in food intake and locomoter activity between Tg and WT mice. In liver, mRNA expression of gluconeogenesis (G6Pase, PEPCK) and lipogenesis related genes (SREBP1c, FAS, ACC, SCD) are reduced in Tg rather than WT mice under HF diet.
Conclusions: Collectively, vaspin improves insulin sensitivity by acting on insulin target organs and may be a new molecular target in the treatment of metabolic syndrome.

NVP-AEB071: Oral and Specific Inhibitor of T Cell Activation for the Prevention of Graft Rejection and the Treatment of Autoimmune Diseases

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Inhibition of T-cell activation is the most efficient way to prevent transplant rejection. Among the immunosuppressants currently on the market, only calcineurin inhibitors (CNIs) inhibit T-cell activation, but their long term use is associated with side-effects (nephrotoxicity and cardiovascular side effects). NVP-AEB071 is the first LMW inhibitor preventing T-cell activation via a calcineurin-independent pathway. NVP-AEB071 inhibits all classical (□, □ and □) and novel □,□,□ and □) protein kinase C isotypes. Herein, we present the case story of NVP-AEB071 including the medicinal chemistry approaches, critical PK properties of the compound, preclinical data including in vivo models in rodents and non-human primates as well as positive proof-of-concept results obtained with NVP-AEB071 in psoriasis patients.

Absorption, Kinetics, Metabolism and Disposition of the Renin Inhibitor Drug Aliskiren

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Background: Aliskiren is the first in a new class of orally active, non-peptide direct renin inhibitors developed for the treatment of hypertension. Over decades, efforts to develop clinically effective renin inhibitors failed, often due to low oral bioavailability. Aliskiren was designed based on crystallographic analysis and molecular modelling. After oral bioavailability had been found in animals, clinical development was initiated and clinical efficacy and safety could be shown. Absolute bioavailability in humans was determined as 2.6%.
Methods: To elucidate the fate of aliskiren in the human body, an absorption, distribution, metabolism and excretion study was performed in healthy volunteers (n=4) with ^{14}C-labeled aliskiren. Single doses of 300 mg aliskiren (2.8 MBq) were administered in a aqueous drink solution. Analysis of biological samples was by liquid scintillation counting and accelerator mass spectrometry for ^{14}C, and LC-MS/MS for aliskiren. Metabolites were analysed using LC-MS/MS and ^{1}H-NMR.
Results: Peak plasma levels of aliskiren (C_{max}) were measured between 2 and 5 h post-dose. Unchanged aliskiren represented the principal circulating species in plasma, accounting for 81% of plasma radioactivity ($AUC_{0-\infty}$), and indicating low exposure to metabolites. Terminal half-lives of aliskiren and radioactivity in plasma were 49 and 44 h, respectively. Dose recovery over 168 h was complete (91.5%), with excretion occurring largely via the faecal route, and mainly in the form of unabsorbed, unchanged drug. Mean urinary excretion was 0.6% of dose. The main metabolic pathways were O-demethylations and subsequent side chain oxidation.
Conclusions: The exact extent of absorption of aliskiren was not determined. Absorption was cautiously estimated to at least 5% but may well have been higher. Absorbed aliskiren was partly metabolized and eliminated mainly via the hepatobiliary route. Aliskiren is a substrate of pgp. Due to the limited extent of aliskiren biotransformation and since aliskiren does not inhibit CYP enzymes to a relevant degree, aliskiren has a low potential for metabolic drug interactions.

Progesterone and its Metabolites have Functional Effects on Processes other than Pregnancy, such as those involving Stress, Affect, and Cognition

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Background: Although progesterone (P$) and its products, such as 5α-pregnan-3α-ol-20-one (3α,5α-THP; allopregnanolone), are typically thought of in their role in maintaining pregnancy, P_4 and 3α,5α-THP can enhance a wide variety of other functional processes. There are data from clinical studies and animal models that 3α,5α-THP can modulate the hypothalamic-pituitary-adrenal axis/stress responses, decrease anxiety or depressive-like behavior, and may even exert some protective effects in neurodegeneration (i.e. epilepsy, Alzheimer's Disease). We have been investigating the effects and mechanisms of progestins using a bioassay, reproductive behavior (lordosis) of female rodents. We have shown that 3α,5α-THP has actions in the midbrain ventral tegmental area (VTA) to modulate the intensity and duration of lordosis and actions in the hippocampus to reduce anxiety-like behavior. Furthermore, 3α,5α-THP increases in midbrain as well as the hippocampus in response to reproductive, as well as stressful/noxious, stimuli. Of interest are the effects of progestins for other hippocampally-mediated processes, such as cognition. **Methods:** We have conducted studies investigating the effects of progestins in the hippocampus in young and aged rodent models to test the hypothesis that 3α,5α-THP can have mnemonic effects. In each, the main endogenous source of P_4 was removed (the ovaries), P_4 or 3α,5α-THP or placebo vehicle was administered to experimental subjects, and cognitive performance was assessed.
Results: We found that young ovariectomized rats or mice, as well as aged mice (which have naturally low progestin levels) administered treatments that enhance 3α,5α-THP (P_4, 3α,5α-THP), but not those that do not enhance 3α,5α-THP (placebo vehicle, medroxyprogesterone acetate) have enhanced cognitive performance. In a transgenic mouse model of Alzheimer's Disease, there were concomitant deficits in hippocampus-mediated processes and 3α,5α-THP evels following long-term P_4 administration, compared to that observed in wildtype mice.
Conclusions: These data suggest that progestins can have functional effects beyond their modulation of reproduction and affective behavior to influence mnemonic processes through actions in the hippocampus.

Bioartificial Human Tissues as Model Systems for Pharmaceutical Target Screening and Drug Development

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Background: In order to translate the findings from basic research into clinical applications, cell-based models need to recapitulate both the three-dimensional (3D) organization and multicellular complexity of an organ. Tissue engineering provides new perspectives for basic and applied research by offering 3D tissue-cultures resolving fundamental obstacles encountered in currently applied 2D and 3D cell-culture systems, including their lack of vascularization. Affording more detailed insight into the complex interactions in tissue differentiation, carcinogenesis, angiogenesis and stromal reactions they may accelerate the progress in design and development of cancer therapies.
Methods: Bioartificial human tissues equipped with a feeding artery, a communicating capillary network and a draining vein were generated by decellularization of porcine jejunal segments and reseeding it with human cells isolated from tissue biopsies. Depending on the intended model system, dermal fibroblasts, hepatocytes, respiratory epithelium, enterocytes or various tumor tissues (carcinoid, pleural mesothelioma, non-small cell lung cancer, lymphangiomatosis) were used for scaffold reseeding following re-endothelialization of the enclosed vascular and capillary network. Tissue differentiation was controlled by histology, immunhistochemistry, cytokine relase, metabolite release, and tissue specific microarrays. In preliminary studies, the metabolic activity of bioartifical human liver-like tissue was characterized.
Results: Co-culture conditions for various cell types were successfully developed to generate bioartificial human skin, liver, airway and eventually intestinal tissue. These tissues could be applied for applied research: I) drug diffusion, II) drug resorption, III) drug penetration, IV) circulatory distribution and V) drug metabolization. Using lymphangioma-tumor tissue, a new tumor cell type could be identified. The bioartificial liver tissue was viable for 3 weeks without histological and functional evidence of dedifferentiation. Biochemical testing revealed stable metabolic activity of the tissue culture. The bioarticial airway model showed coordinated cilial activity for more than two weeks.
Conclusions: Bioartifical human tissues represent versatile model systems for pharmaceutical target screening and drug development, complying with the European Union's REACH regulations. However, their effective potential in expediting drug development and testing is subject of ongoing research.

TNF mAbs: Magic bullets for tuberculosis?

WALLIS RS

Pfizer

TNF is central to the pathogenesis of chronic inflammation but is required for host defenses against granulomatous pathogens such as M. tuberculosis. The TNF antibodies infliximab and adalimumab are differentiated from soluble TNF receptor (etanercept) by their efficacy against granulomatous forms of inflammation (e.g., Crohn's disease), and their 5-10 fold higher high risk of reactivating latent M. tuberculosis infection. We examined the basis of these observations by studying the effects of TNF blockers on TB-induced gene expression in whole blood cultures of 6 healthy tuberculin reactive volunteers. 791 genes were significantly up or downregulated by Mtb, of which some were downregulated by TNF blockers: 75 by infliximab, 60 by adalimumab, and 40 by etanercept (P<.001). All 40 of the etanercept-affected genes were also affected by infliximab and/or adalimumab. Among the genes specifically inhibited by TNF mAbs were IFN□, INDO, OAS3, and STAT1. Inhibition of these key immune response genes in TB granulomas may account for the excess risk of reactivation posed by anti-TNF antibodies.
The granulomatous response to Mtb contains an infection that cannot be otherwise readily eradicated. However, in TB, this response causes clinical disease manifestations and lung pathology. Granulomas also interfere with the microbiologic response to therapy, preventing drug access and delaying sterilization of sequestered dormant bacilli. The deleterious effects of the granulomatous host response in TB are most apparent in patients with paradoxical worsening precipitated by withdrawal of TNF blockade, in whom TNF-dependent inflammation is restored. We present two such cases of life-threatening paradoxical worsening treated by resuming or starting TNF mAb therapy. In both cases the clinical therapeutic response to anti-TNF therapy was strikingly rapid. In neither case was the microbiologic response to TB therapy adversely affected. Further studies of adjunctive anti-TNF therapy in TB are warranted. Current recommendations for the routine withdrawal of TNF blockade in patients who develop TB should be reconsidered.

Chronic Levonorgestrel Treatment in a Macaque Species (Macaca sylvanus): effects on sex steroids and secondary sex characteristics

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Background: Administered levonorgestrel enables contraception under normal cyclicity of the ovaries and does not suppress the development of cyclic perineal swellings. This study investigated the influence of levonorgestrel on fecal gonadal steroid excretion rates and the expression of perineal swelling size.
Methods: Two groups of Barbary macaque females were observed: Twenty-four implanted individuals under semifree conditions and five non-implanted individuals under caged conditions. Eight of the implanted females had large expressions of the perinea and sixteen reduced. The non-implanted group had no perineal swellings. To determine the individual steroid excretion rates an enzymeimmunoassay for fecal samples was established.
Results: Estradiol excretion rates did not differ in distinctively swollen implanted females, but were increased when compared to non-implanted individuals (df=2; p=0.0002). Implanted females with large perineal swellings had lower progesterone concentrations in the feces compared to individuals with reduced swellings and did not differ from the non-swollen group (df=2; p=0.054). Females with large perineal swellings showed a higher calculated estradiol to progesterone ratio index than the other groups (df=2; p=0.0005). Non-implanted individuals showed increased testosterone excretion rates (df.=2; p=0.0001).
Conclusions: The results indicate a positive relationship between the perineal swelling size and levonorgestrel implantation. The ratio of fecal estradiol and progesterone titers can be judged as an endocrine indicator for the expression rate of perineal swelling size.

Antimicrobial Resistance in South Africa: 'Craving for a Magic Bullet'

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Background: Antibiotic resistance (AR) is a problem in South Africa owing to their wide spread use. Several studies have shown that resistance is of concern in hospitals due to nosocomial infections, but community acquired infections have also exhibited change in the resistance pattern. Also, antibiotic resistance in public health facilities appears to be different from that in private institution. Unfortunately, this information is reported in different articles. Here, we undertake a review and analysis of these reports with a hope that it will improve accessibility and utility in the fight against AR.
Methods: A search for relevant abstracts during 1966 to 2007 was done on Medline database using search terms that included: South Africa, antibiotic, resistance and prevalence. The full length articles of the relevant abstracts were evaluated for inclusion in the review. Other relevant articles were obtained from local journals. Results were reported as average prevalence (%) of AR with time or to multiple antibiotics.
Results: Thirty-four abstracts were recovered and, of these, 9 articles qualified to be used in this review. In survey of 7 academic hospitals (2001-2004), the prevalence of MRSA was 46.4%, while in a 6 month study of 12 private laboratories, it was 36%. The prevalence of Penicillin-resistant pneumococci (PRP) was 45% in 1997, having risen from 31.3% in 1996. In another report, the prevalence of PRP in community acquired Lower Respiratory Tract Infections increased from 29.4% in 1996 to 35.8% in 1997, while the prevalence of β-lactamase producing H. influenzae in one academic hospital rose from 33% in 2001 to 40% in 2003. Regarding multiple AR the prevalence was gentamycin 45.6% and ciprofloxacin 11.3%, while that of Ps. aeruginosa it was; meropenem 42%, imipenem 45%, cefepime 53% and ciprofloxacin 46%.
Conclusions: 1) The prevalence of AR in South Africa is very high (> 20%), on an upward trend, and multiple AR is on the increase. 2) There was increased use of the newer antibiotics such as moxofloxacin, levofloxacin, telithromycin for community acquired infections, and the meropenem, linezolide and vancomycin for nosocomial infections, and this has lead to emergence of resistant strains to these drugs. 3) There is a need for new and more effective antibiotics before we run out of options. In effect, South Africa is craving for a Magic Bullet.

Therapeutic protein engineering via the incorporation of non□natural amino acids

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Incorporation of unnatural amino acids into recombinant proteins represents a powerful tool for protein engineering and protein therapeutic development. The presence of the unnatural amino acid in recombinant protein enables site-specific modification of protein at these unnatural amino acids. For example, site-specific pegylation of protein extends the in vivo half-life of therapeutic protein, while site-specific conjugation of antibody to toxin has long been regarded as "guided missile" in treating cancer patients. In this study, we have engineered human interferon-beta protein with two unnatural amino acids, L-azidohomoalanine (AHA) and L-homopropargylglycine (HPG). We report the effects of the penultimate residue (the residue after the initiator Met) on the processing of AHA and HPG, at the N-terminus of recombinant human interferon-beta in *E. coli*. We have identified specific amino acids at the penultimate position that can be used to efficiently retain or remove N-terminal AHA or HPG. Retention of N-terminal AHA or HPG can be achieved by choosing amino acids with large side-chains (such as Gln, Glu and His) at the penultimate position, while Ala can be selected for the removal of N-terminal AHA or HPG. We also report site-specific pegylation at AHA site and characterization of pegylated IFN-beta protein.

The treatment of patients with multi-drug resistant cavitary pulmonary tuberculosis with gelatin containing anti-tuberculosis medicines by fiberbronchoscope

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Background: The situation of tuberculosis (TB) and drug resistance were severe in China. The emergence of multidrug-resistant *M. tuberculosis* (MDR-TB) strains has made the treatment of TB (specially cavitary pulmonary TB) become more difficult. The standard chemotherapy for them needs longer curable duration. The goal of this paper is to describe the effect of local treatment of MDR-cavitary pulmonary TB by fiberbronchoscope.
Methods: Two hundredes and twenty-three patients with MDR- cavitary pulmonary TB were treated with the same standard chemotherapeutic scheme. They were divided into two groups: control group and interposition group. The control group (containing 37 patients) was treated only with medication, while interposition group (containing 186 patients) was treated in combination with gelatin containing Dipasic, streptomycin, Pyrazinamide, and Levofloxacin by fiberbronchoscope. The clinical curative effects were observed for 6 months.
Results: In the interposition group at the end of six-month therapy, the sputum negative conversion rate was 73.1%, the lesion marked absorption rate was 85.4%, and cavity closing rate was 81%, which were higher than that of the controls (51.3 %, 56.7% and 51.4%, $P < 0.05$). The time of sputum negative conversion was 36.3 days in interposition group, which was shorter than that of the controls (61.8 days, $P < 0.01$). There was not severe adverse reaction in interposition group.
Conclusion: The efficiency of the chemotherapy combined with local treatment by fiberbronchoscope is better than the routine chemotherapy for the MDR-cavitary pulmonary TB.

On the Delivery of Carmustine, Paclitaxel, and Etanidazole to Brain Tumors: An integrated study on the novel fabrication methods of pharmaceutical particles and 3-D computer simulations for chemotherapy and radiotherapy applications

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Conventional treatment of brain tumor involves excising the main part of the tumor in conjunction with post-surgery treatments, such as chemotherapy, radiotherapy, or a combination of both. One of the difficulties associated with such treatment is that the tumor tissue cannot be completely removed without the risk of cutting off neighboring healthy normal tissue. In addition, the tumor that remains behind is capable of rapid multiplication and encroachment into nearby healthy tissues. In traditional chemotherapy, anti-cancer agents are delivered systemically to the malignant site via systemic administration to kill the cells. However, the presence of high interstitial pressure within the tumor's centre further poses severe problems to chemotherapy via systemic administration [1]. For effective radiotherapy, the patients are subjected to a scheduled course of repeated radiation to destroy the remnant tumor cells. The present study is undertaken to investigate the optimal way of drug administration to tumors via the combination of chemotherapy and radiotherapy. The study consists of two major parts: In the first part, controlled drug release devices are developed in the dosage forms of microparticles and disc wafers of biodegradable polymers PLGA [poly(d,l-lactide-co-glycolide) and PLA [poly(l-lactide)]. In the second part, computational fluid dynamics (CFD) simulations are used to analyze the spatial and temporal variations of drug concentration. The efficiencies of different ways of drug administration are compared among a variety of anticancer and radiosensitizer agents and drug-polymer systems. The first part of this study illustrates the fabrication of PLGA polymeric particles by electrohydrodynamic atomization (EHDA) for applications in sustained delivery of anticancer drug - paclitaxel to treat C6 glioma cells in vitro and in vivo. The differential scanning calorimetry study indicated that paclitaxel could be either in an amorphous or disordered-crystalline phase of a molecular dispersion or a solid solution state in the polymer matrix after fabrication. The X-ray photoelectron spectroscopy result suggested that some amount of paclitaxel could exist on the surface layer of the microparticles. The encapsulation efficiency was around 80% and more than 30 days in vitro sustained release profile could be achieved. Cell cycling results suggested that paclitaxel after encapsulation by EHDA could keep its biological function and inhibit C6 glioma cells in G2/M phase. The cytotoxicity of paclitaxel-loaded biodegradable microparticles to C6 glioma cells could be higher than Taxols(r) in the long-term in vitro tests evaluated by MTS assay. The drug delivery devices developed by EHDA in this study could be promising for the local drug delivery to treat malignant glioma. This work also examines the in vivo drug release profile and anti-tumor responses of BALB/c nude mice, bearing C6 glioma tumors subcutaneously, to treatments by PLGA microspheres, microparticles and discs-delivering Paclitaxel and Etanidazole. The in-vivo experimental results are used to correlate the efficacy of treatment to in vitro release profiles from the various formulations. Our study demonstrates that radio-sensitizing effects during irradiation could be achieved by double burst profiles from Etanidazole-loaded discs, when compared to controls 17 days after implantation despite the short half-life of Etanidazole (1.4 h) in vivo. These results also showed inhibited tumor growth on tumor volumes of 59%, 65% and 70% over the blank placebo groups after 21 days of tumor growth for spray-dried microspheres, EHDA microparticles and spray-dried discs, respectively [2,3,4].
The second part of this paper further reports the development of three-dimensional computer simulation models to study the effect of various factors on the delivery of carmustine, paclitaxel and etanidazole to brain tumors for integrated chemotherapy and radiotherapy applications. The study yields information on the efficacy of various delivery methods, and the optimal location of polymer implantation. Two types of drug deliveries, namely, systemic administration and controlled release from polymers, were simulated to predict the temporal and spatial variation of drug distribution. The simulation was carried out using CFD simulations with the model geometry constructed from magnetic resonance images (MRI) with reference to the Gliadel(r) wafers application [5]. The simulation results show that polymer-based delivery provides higher mean concentration, longer carmustine exposure time and reduced systemic toxicity than bolus injection. Polymer implanted in the core gives higher concentration of drug in both the core and viable zone than the polymer in the viable zone case. The implantation of carmustine/ polymer matrix at the lumen of the viable zone immediately following the surgical removal of 80% of the tumor may be an effective treatment for the chemotherapy of brain tumors. Removal of the tumor core by surgery and subsequent insertion of drug-carrying polymers in the resection cavity may further improve the treatment. The operation establishes a favourable pressure gradient towards the centre of the tumor and thus creates flow reversal immediately after the operation. The presence of post-surgery edema increases the interstitial pressure and fluid velocity, thus causing higher relative toxicity in the surrounding normal tissues. The CFD model is also extended to radiotherapy applications for analyzing the penetration depth of Etanidazole in tumor impanted with PLGA wafers in the resected cavity [6,7,8,9,10].

Nucleotide Excision Repair (NER) in cisplatin-induced cellular responses

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Cisplatin is an important anticancer drug that has been used in the treatment of many types of cancer. Cisplatin binds to the DNA template to form both intra- and inter-strand crosslinks (ICL). Recognition of this ICL damage can eventually lead to apoptotic cell death, which is an important mechanism for cisplatin in its anticancer action. Nucleotide excision repair (NER) plays an important role in removing DNA damage generated by cisplatin. The results obtained from our studies reveal that the DNA damage recognition signal initiated by XPC protein, an important DNA damage recognition protein for the NER, is essential for cisplatin-induced cellular responses such as cell cycle arrest and apoptosis. The DNA damage recognition signal of XPC protein is required for cisplatin-induced p53 phosphorylation, while the XPC-TFIIH complex formation plays a key role in the p53 phosphorylation. The phosphorylated p53 protein further regulates transcriptions of a series of downstream target genes to result in cell cycle arrest and/or apoptosis. Furthermore, the process of cisplatin DNA damage by NER also causes activation of the ATM protein, which then induces transcription of an important anti-apoptotic *bcl-xl* gene through NF-□B, resulting in increased accumulation of Bcl-x(L) and subsequent cell survival under cisplatin treatment. Therefore, the NER possesses two very distinct roles in the cisplatin-induced cellular responses: (1) the DNA damage recognition signal initiated by the XPC protein is essential for cisplatin-induced cell cycle arrest and apoptosis; and (2) the NER process of cisplatin DNA damage is required for ATM activation and cell survival under cisplatin treatment. Therapeutic manipulations that enhance the DNA damage recognition-induced cell cycle arrest and apoptosis and repress the NER process-mediated ATM activation and cell survival will significantly improve the effectiveness of cisplatin in anticancer treatment and reduce tumor cell resistance to cisplatin.

Assessing the Metabolic Liabilities of Aromatic Amines using In Vitro Metabolism and Mass Spectral Techniques

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Background: The purpose of this study was to establish a combination of in vitro metabolism and mass spectral techniques that could potentially be used to assess metabolic liabilities of aromatic amines in mutagenicity. An attempt was made to correlate the Ames results with formation of the deoxyguanosine adducts.
Methods: 2-Aminofluorene (2AF) was used as a model compound that was bioactivated in the presence of rat (aroclor induced) liver S9 fortified with NADPH and N-acetyl CoA. Deoxy-guanosine (dG) was used as a nucleophilic trapping agent. A covalent adduct of 2AF-dG, the formation of which depended on the presence of CYP1A2 and NAT2 in the incubations, was characterized by LC/MS. By employing the technique 11 model compounds, their mutagenicity have been evaluated by Ames assay, were studied.
Results: 1) The mass spectral data suggested the covalent binding of aniline nitrogen of 2AF with C8 of deoxyguanosine, consistent with the bioactivation pathways described for this compound in the literature, which suggests that the technique is valid in trapping bioactivated aromatic amine. 2) Quantitative LC/MS analysis of the deoxy-guanosine adducts formed with the 11 target compounds demonstrated that the correlations were 0.85 and 0.54 in ranking the reactivity of the model compounds by the peak area of dG of LC/MS vs by revertrants/nmol of TA100 and TA98 of Ames assay, respectively. 3) The quantitative data also demonstrated that electron withdrawing substituents and stereo hindrance substantially decreased the levels of the trapped adduct. These preliminary results suggest that this technique could potentially be applied to guide synthetic efforts in mitigating bioactivation of such compounds.

Effect of Genetic Polymorphisms in ABCG2 (BCRP) on Inhibition and Potential Drug Resistance

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ABCG2 (Breast cancer resistance protein, BCRP, MXR) is an adenosine triphosphate-binding cassette transporter that effluxes drugs and metabolites out of cells such as anticancer compounds. Single nucleotide polymorphisms (SNPs) for ABCG2, V12M (g34a) and Q141K (c421a), have a high frequency in the population. In addition, Q141K is reported to significantly affect the pharmacokinetics of diflomotecan. Further, an amino acid change at position 482 (R482G, a1444g) that occurs in some drug-resistant cell lines has differing substrate affinity compared to the wild type (WT) protein. However, R482G has not been identified in normal population to date. All of these SNPs are known to lead to changes in protein level or activity of the transporter. Therefore, understanding role of the polymorphisms on changes in substrate and inhibitor affinity is key to unraveling their impact on clinical outcomes To explore the transport kinetics of these SNPs against various ABCG2 substrates and inhibitors, we have created cell lines that functionally express ABCG2 WT, V12M, Q141K or R482G. The expression levels of WT and polymorphic alleles are similar based on both western blot and semi-quantitative RT-PCR. Confocal microscopy demonstrated that all the ABCG2 variants were localized to the cell membrane. A fluorescent inhibition assay was developed and was utilized to measure IC50 against 13 compounds for ABCG2 WT and variants. The results showed that SNPs can have different IC50 against certain inhibitors, indicating that SNPs can play a potential role in drug resistance, and when using a drug that is an ABCG2 substrate/inhibitor, the impact of genetic variation should be considered.

Magic bullet and magic shield: a new strategy to target Survivin in human cancers

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Background: Survivin belongs to the Inhibitor of Apoptosis Protein (IAP) family and plays a critical role in modulating the spindle checkpoint control during mitosis. verexpression of Survivin is commonly found in various forms of human cancers and is implicated in increasing aneuploidy, as well as resistance to genotoxic agents. Conversely, down-regulation of Survivin may sensitize cancer cells to genotoxic treatment. The goal of this study is to demonstrate that targeting Survivin can be used as a strategy to treat human malignancy.
Methods: Small molecule Survivin anatgonists have been developed based on our recently demonstrated Cavity-Induced Allosteric Modification (CIAM) approach, which evaluates surface cavities and clefts as potential binding sites for pseudoallosteric molecules. The binding properties of the Survivin-targeting molecules have been evaluated with isothermal titration calorimetry (ITC). The biological activities of these molecules have been examined with cell-based assays and with in vivo animal models.
Results: Our in silico screen identified small molecules that target the Survivin protein at a site important for its function. By site directed mutagenesis and isothermal titration calorimetry analysis, our data indicate the Survivin-targeting molecules bind to intended interface. In addition, the Survivin anatgonists elicit mitotic arrest and causes apoptosis in a cell cycle dependent manner. Finally, we have demonstrated that the Survivin-targeting molecules can inhibit tumor growth in the mouse xenograft model.
Conclusions: 1) We have identified small molecules that target the dimerization interface of Survivin. 2) The Survivin-targeting molecules arrest cells in the early stage of mitosis. 3) Disruption of Survivin functions by the small molecules causes apoptosis in a cell cycle dependent manner. 4) The Survivin-targeting molecules inhibit proliferation of cancer cells in vitro and tumor growth in vivo.

Functional characterization of outer membrane proteome in response to antibiotic resistance

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Background: The worldwide emergence of antibiotic-resistant bacteria poses a serious threat to human health. To understand the mechanisms of the resistance is extremely important to the control of these bacteria. A line of evidence has indicated that four mechanisms are probably involved in the antibiotic resistance. They are modification or hydrolysis of enzymes, modification of targets, activation of efflux pump systems, and reduction of outer membrane (OM) permeability. The permeability and the pump systems are mainly controlled by OM proteins in Gram-negative bacteria. The decrease of OM permeability prevents the influx of antibiotics, and the activation of the efflux pump systems pumps the noxious small molecule substances out of cells. However, little is known about OM proteome and the two-component regulating system of these proteins involved in the NA resistance of *E. coli*. Recently, we have systematically investigated altered OM proteins of *E. coli* in response to ampicilin, kanamycin, tetracycline, nalidixic acid, streptomycin resistance.
Methods: 1) A differential OM sub-proteome in response to these antibiotics is achieved with the use of 2-DE proteomics and Western blotting methods; 2) Key OM proteins in response to these antibiotics are determined by analysis of functional characterization of the altered OM proteins using their genetic modified strains; 3) Two-component system regulation and a network of the altered OM proteins are investigated using Western blotting and/or bacterial survival capability analyses.
Results: Several differential expressed proteins are determined in each of the antibiotics tested: Up-regulated FimD, Tsx, OmpW, OmpC and TolC, and down-regulated NlpB for ampiciline; up-regulated FimD, Tsx, OmpW, OmpC and TolC, and down-regulated NipB and LamB for tetracycline; up-regulated TolC,OmpT and LamB, and down-regulated FadL, OmpW and Dps for streptomycin (SM). up-regulated TolC, OmpT, OmpC and OmpW,and down-regulated FadL for nalidixic acid (NA). Up-regulation and down-regulation of OmpC were observed respectively, in NA-R and Δ*ompF*, and Δ*envZ* and Δ*ompR* cultured with or without NA. Meanwhile, OmpC level in Δ*envZ* and Δ*ompR* was significantly lower in cultures with 1/2 MIC NA than the cultures without NA. the interaction network among the altered OM proteins was characterized based on effect in absence of each of the six altered proteins on the other five in SM-resistant genetic modified strains with gene deletion. Of the six altered proteins, TolC mainly regulates other proteins, and OmpT, LamB and Dps may be middle factors at a center of the network.
C**onclusions:** 1) Alteration of OM proteins in resposne to antibiotics is related to chemical characteristics of the antibiotics used, but the OM proteins shared by all antibiotic-resistant strains exist., suggesting universal and specific antibiotic-resistant mechanisms for OM proteins against antibiotics. 2) EnvZ/OmpR two-component system plays an important role of in the regulation of NA resistance. 3) the present study demonstrates that a regulating network exists among the altered OM proteins. In the network, TolC mainly regulates other proteins, and OmpT, LamB and Dps may be middle factors at a center of the network. These results may suggest that a biological feedback network exists in *E. coli* with SM resistance.
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New multifunctional pharmacophores and biocompatible nanocomposites for targeting drug delivery and cancer diagnosis

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It is well known that the failure of chemotherapy to the malignant tumor is usually induced by multidrug resistanc (MDR),and the development of effective anti-MDR agents for efficient drug delivery plays an important role in the tumor therapy. Multidrug resistance (MDR) is the ability of disease-causing organisms to withstand a wide-variety of structurally and functionally distinct drugs or chemicals. MDR involves the expression of membrane proteins
which mediate the active extrusion of drugs from the cell and it is a major limiting factor to the chemotherapy of cancer. Because of the importance of MDR in clinical oncology, an extensive search for the new MDR reversal agents is still an unceasing challenge. Many compounds known to have other pharmacological sites of action initially were used to reverse MDR in cancer cells grown in culture and several underwent pilot clinical trials. A large
number of small molecules capable of modulating P-gp mediated MDR have been described in the literature. Among the potentially pharmaceutical functional molecules, the remarkable chemical and thermal stability and hydrophobic character allow the carborane to be used as a promising pharmacophore in biologically active moleculesfor anti-drug resistance and anti-cancer application. Our recent studies indicate that when co-administered with a cytotoxic agent, these nontoxic modulators enhance net accumulation of relevant cytotoxic drugs within the tumor cells. In recent years, nanomaterials, which show unique physical and chemical properties, have attracted much attention in various fields and have been widely applied in biological and biomedical engineering. In this report we will pre-sent some of our recent research progress in combining the design of multifunctional biocompatible promising pharmacophore and nanomaterials with targeted and efficient drug delivery system. These studies demonstrate that with the combination of multifunctional pharmacophore or nano-interface, we can realize the synergistic effect on the efficient cytotoxicity suppression in drug sensitive and drug resistance cancer cells and enhance intracellular drug accumulation of anticancer drug into target cancer cells. Meanwhile, our observations also indicate that due to the convenient surface functionalization, the biocompatible nanomaterials can play versatile roles for fabrication of high sensitive probes and biosensors in cancer diagnosis, which show great promise in the design of point-of-care-testing devices in the future.
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Temozolomide Acid Hexyl Ester (TMZA-HE) as a Topical Bullet for Skin and Cervical Cancers

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Background: Temozolomide (TMZ) has been considered as the first choice for treatment of glioma; however, its phase III trials failed to show a significant improvement over dacarbazine in treatment of melanoma. This failure may be due to its virtual insolubility in organic and aqueous media, resulting in inadequate drug distribution into skin. A group of temozolomide acid (TMZA) esters were designed in order to 1) investigate if the esters are prodrugs with the ability to penetrate through skin; 2) verify bioactivities of the ester prodrugs against cancer cell lines and in an animal model; 3) explore topically applicable formulations of the ester prodrug for skin and other cancers.
Methods: TMZA methyl to octyl esters were synthesized and fully characterized. NMR was applied in monitoring enzymatic hydrolysis of the ester *in vitro*. The skin penetration potency of the esters was assessed with silicone membrane, rat skin and human skin. Bioactivities of TMZ, TMZA and TMZA esters were measured against cancer cell lines. Developing a formulation for TMZA-HE is challenging because its sensitivity to a nucleophilic attack restricts the use of many popular excipients. Bioactivities of a TMZA-HE microemulsion were assessed in BALB/c nude mice, inoculated with MV3 human melanoma, A431 human skin Basal cell carcinoma, HCT116 human colon carcinoma and HeLa4 human cervical cancer.
Results: *In vitro* the TMZA ester was enzymatically hydrolyzed into TMZA rapidly under incubation condition. The ester prodrugs showed superior ability for skin penetration and readily converted into TMZA within the skin. TMZA-HE demonstrated an adequate balance of skin permeation and retention. IC_{50} of TMZ, TMZA and TMZA-HE are equal for cancer cell lines. A microemulsion formulation of TMZA-HE with a satisfactory shelf-life was developed using a novel excipient. Growth of MV3 human melanoma and HeLa4 human cervical cancer in BALB/c nude mice was significantly inhibited by the formulated TMZA-HE product.
Conclusions: 1) TMZA esters are prodrugs of TMZA (TMZ) with skin penetrating potency. 2) The adequate balance of skin permeation and retention of TMZA-HE warranted promising further developments. 3) The topically applicable TMZA-HE formulation showed promise for treatment of melanoma and cervical cancer.

From Bark to Bullet: A Personal History of the Discovery and Development of Taxol

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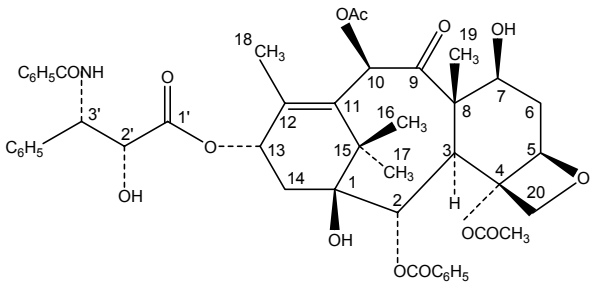
Taxol is a secondary metabolite obtained from the wood bark of Taxus brevifolia, found in the Pacific Northwest coastal region of the United States. It was isolated by the bioassay-guided fractionation of the crude plant material. The structure of Taxol was established by single crystal x-ray analysis. Taxol has a unique mechanism of antitumor activity. It inhibits cancer cell growth via stabilization of microtubules.

Currently, Taxol is approved for clinical use in the USA by the FDA for the treatment of refractory ovarian, breast, and non-small cell lung cancers and Kaposi's Sarcoma. This presentation will describe the 30-year efforts which transformed this compound from an interesting plant secondary metabolite to a life saving chemotherapeutic agent.



Taxol

Increased PGC-1□ expression exhibits protective effects against age-related neurodegenerative and metabolic disease processes

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Background: It is generally accepted that mitochondrial function declines with age, as evidenced by the increased level of cellular oxidative stress due to increased mitochondrial production of reactive oxygen species (ROS). It is also widely accepted that age-related alterations of mitochondrial function constitute a major component of the aging process. The transcription co-activator peroxisome proliferator-activated receptor □ coactivator-1□ (PGC-1□) is known to be a powerful stimulator of mitochondrial biogenesis. We were therefore interested in determining if stimulation of mitochondrial biogenesis would lead to an improvement in mitochondrial function and thereby modulate the aging process.
Methods and Results: To this end a unique transgenic animal model has been developed in which the human PGC-1□ gene has been inserted into the C57Bl6/J mouse genome. Ubiquitous 2 to 3 fold increases in the expression of human PGC-1□ mRNA and protein was obtained. These changes were accompanied by 2 to 4 fold increases in mRNA and protein of transcription factors and other proteins associated with mitochondrial biogenesis (mTFA, COX II, etc.). Unexpectedly, we found that the transgenic animals exhibited an improvement in the glucose tolerance response. Use of the euglycemic-hyperinsulinemic clamp technique demonstrated that this is due to increased skeletal muscle insulin sensitivity. Another unexpected finding was that cross-breeding the PGC-1□ animals with the G93A mouse model of amylotrophic lateral sclerosis (ALS) exhibited a rescuing effect as evidenced by the extension of life span and marked improvement in RotoRod performance. This finding is consistent with the reports that increased expression of PGC-1□ exhibits a rescuing effect in a murine model of Huntington's disease.
Conclusions: Alterations of PGC-1□expression, or activity, may therefore provide a mechanism for altering the progress of a metabolic disease such as Type II diabetes mellitus as well as in neurodegenerative diseases such as ALS and Huntington's disease. (Supported by NIH grant AG028294)

"The legacy of Paul Ehrlich To antimalarial chemotherapy"

WARHURST D

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Review: Ehrlich's work forms an essential mechanistic background for current views of antimalarial chemotherapy. This is exemplified here by the 4-aminoquinoline chloroquine [CQ], and the arylaminoalcohol quinine [(-)QN] and related compounds. These are hydropathic weak bases which become concentrated in the acid content of the lysosome (digestive vacuole:DV) of blood stage malaria parasites by protonation to CQ2H+ or QNH+ (haptophore effect). These magic bullets bind a haematin target, released during digestion of host haemoglobin, and they prevent detoxication to malaria pigment. (toxophile effect). In CQ-resistant (CQ-R) Plasmodium falciparum, since target haematin cannot change, access of drug is diminished. In CQ-R, PfCRT protein in the DV membrane differs from CQ-S by 2 residue changes, K76T in transmembrane helix 1 (TMH-1), which removes a positive charge in a putative CQ2H+ efflux channel, and A220S in TMH-6. The effect of K76T in TMH-1 is increased in SE Asian and related African isolates by introduction of a negative charge (N to E) on 75, which with 72 and 76 lines the channel, and M74I, hydropathy increase on the membrane side. TMH-1 in many Asian, Oceanian and S. American CQ-R isolates shows only C72S in addition to K76T, giving increased polarity, reducing the resistance-reversing effect of verapamil and enhancing resistance to desethylamodiaquine (D-AQ).
CQ-S P. falciparum is more sensitive to (-)QN diastereomer (+) quinidine than to (-)QN. In parasites with experimental replacements of PfCRT K76, this differential effect is maintained: (T), (N), or reversed: (I), and this also applies to in vitro QN-verapamil potentiation seen in CQ-R, indicating that the QN magic bullet has a stereochemical interaction with a second target, the PfCRT channel. The pH-modulated lipid distribution coefficient LogD (from pKa values and LogP) has been important in understanding how more hydropathic 4-aminoquinolines such as AQ and D-AQ are able to retain activity in CQ-R. They show an increase in LogD giving higher concentrations in lipid and allowing binding to the more hydropathic lining of the CQ-R efflux channel. The bis 4-aminoquinoline piperaquine, effective in CQ-R, achieves a LogD (pH4.8) of 0.97 ([medium]: [vacuolar lipid] = 1:973492) while retaining a high concentration in lysosomal water ([medium]: [vacuolar water] = 1:104378) because the number of haptophore-like protonatable amino groups on the molecule is increased from 2 to 4.

Vascular Directed Tumour Therapy by a Novel Pyrazole that Inhibits the Ras-Net (Elk-3) Pathway and Affects Microtubules

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Background: The Growth Factor-Ras-Erk signalling pathway is frequently perturbed in human cancers, and is a target for the development of tumour therapies. The Ras-ERK signalling pathway targets various cellular effectors, including the Net (Elk-3/SAP-2/Erp) transcription factor, which is phosphorylated and activated by ERK, and is involved in wound healing, angiogenesis and tumour growth.
Methods: A cell based screen for small molecule inhibitors of Ras activation of Net transcriptional activity was used for a high throughput screen for small molecule inhibitors of the pathways. Selected molecules were tested for their activity, specificity and mechanisms of action using in-vitro and in-vivo molecular and cellular based approaches.
Results: We identified a novel pyrazole XRP44X. XRP44X inhibits FGF-2 induced Net phosphorylation by the Ras-ERK signalling upstream from Ras. It also binds to the colchicine-binding site of tubulin, depolymerises microtubules, stimulates cell membrane blebbing and affects the morphology of the actin skeleton. Interestingly, Combretastin-A4, that produces similar effects on the cytoskeleton, also inhibits FGF-2 Ras-Net signalling. This differs from other classes of agents that target microtubules which have either little effect (Vincristine), or no effect (Docetaxel and Nocodazole), on the Ras-Net pathway.
Conclusions: XRP44X inhibits various cellular properties, including cell growth, cell cycle progression and aortal sprouting, similar to other molecules that bind to the tubulin colchicine site. XRP44X has the potentially interesting property of connecting two important pathways involved in cell transformation. It is an interesting molecule for the development ofcancer treatment.

Targeted Therapy of Human Neuroblastoma Cells using Auger Electrons of Indium-111-labeled N-myc Antisense Oligonucleotide

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Background: Auger electrons may destroy nucleic acids in the close vicinity, and thus targeted therapeutic effect of Auger electrons of indium-111 (In-111)-phosphorothioate antisense oligonucleotides on human neuroblastoma cells with the overexpression of N-MYC was studied.
Methods: Human neuroblastoma cells of SK-N-DZ (5 X 10^6 cells) were treated for 20 hrs with cationic reverse-phase vesicles encapsulating In-111-antisense oligonucleotides (AS) (average specific radioactivity of vesicle of 40 MBq/2 nmol of oligonucleotides/μmol of total phospholipids) with average diameter of 250 nm. The expression of N-MYC and proliferation of the treated tumor cells were investigated as well as the existence of In-111-AS hybridized with intracellular N-myc mRNA. Tumorigenecity o the treated tumor cells was examined in nude mice. As a control, non-radiolabeled AS or In-111-sense oligonucleotides (S) were employed.
Results: The In-111-AS hybridized with intracellular N-myc mRNA were detected from the treated tumor cells at 12 and 24 hrs after initiation of the treatment. Reduction in the expression of N-MYC (average 27% to control) and inhibited cell proliferation (average 59% to control) were shown in cells at 48 hrs after treatment of In-111-AS.The N-MYC-suppressed cells however continued to produce tumor although a significant decrease in average weight of tumors was demonstrated in intraperitoneal cavity of nude mice. Neither of non-radiolabeled AS nor In-111-S caused any effect in tumor cells.
Conclusions: Auger electrons of In-111 in the close vicinity of their target N-myc mRNA may cause reduction of cell proliferation rate of human neuroblastoma cells with the overexpression of N-MYC, and In-111-antisense oligonucelotides could potentially be a tool for internal molecular radiotherapy at a level of mRNA of a tumor cell.

The Tissue Specific Choice of Anti-caner Drugs on the Basis of Polyamine Level

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Background: Polyamines (putrescine:put, spermidine:spd and spermine:spm) are essential to neoplastic cell growth. Therefore, analysis of the effects of the anti-cancer drugs on the polyamine concentration of individual tissues of intact rats provides tissue specific treatment of the drug on the basis of polyamine level and could improve the management of cancer patient by allowing development of new strategies of the drug use that targets the tumor-bearing tissues.
Methods: The experimental groups of rats (7) received intraperitoneal injections of the drugs and volume of a solution successive daily for 5 days. All rats were euthanized with enflurane on the sixth day, and the tissues were immediately removed, weighed and kept in 2.0 ml of an aqueous 10% trichloroacetic acid solution (TCA) containing 0.1 mmol/L 1,4-diaminohexane as an internal standard in an ice bath. Each tissue was homogenized and then centrifuged at 2500 rpm for 15 min. The supernatants were washed twice with 5 ml diethyl ether to eliminate the TCA in the water layer. Determination of polyamine concentration in the water layer was carried out using HPLC and the amount of each polyamine was calculated from the peak area relative to the internal standard 1,6-diaminohexane
Results: All polyamines in 5-FU- and ara-C-treated small intestine and 5-FU-treated seminal vesicles, and spd and spm in 5-FU-treated prostate and testis, ara-C-treated stomach, adriamycin-treated seminal vesicles and large intestine and cisplatin-treated seminal vesicles of intact rats increased. On the other hand, all polyamines in the adriamycin-treated heart, methotrexate-treated thymus and spleen, cyclophosphamide-treated prostate, seminal vesicles, thymus, spleen, kidney, heart and small intestine of rats, and etoposide-treated thymus and put and spd in adriamycin-treated prostate, testis and thymus and in etoposide-treated skeletal muscle and lung decreased. At the regional brain, all polyamines in etoposide-treated cortex decreased, and spd and spm in etoposide-, ranimustine- and nimustine-treated hippocampus, cortex and corpus striatum of rats decreased, respectively.
Conclusions: (1) Anti-cancer drugs, adriamycin, methotrexate, cyclophosphamide and etoposide is suitable to the treatment of the cancers bearing the skeletal muscle, thymus and spleen, small intestine, prostate, seminal vesicles, thymus, spleen, kidney and heart, and thymus, respectively. (2) 5-FU and Ara-C, respectively, should not use the treatment of the cancer bearing the seminal vesicles and small intestine, and the small intestine because of increase of polyamines that stimulate the growth of the cancer cells. (3) Etoposide is suitable to the treatment of the cancers bearing the hippocampus and cortex, and ranimustine and nimustine to the cortex and corpus striatum, respectively.

When More is Not Necessarily Better: Interdisciplinary Inquiry into the Implications of U-Shaped Dose Responses for Personalizing Anticancer Interventions

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Background: The perception that is pervasive among the public is that, when it comes to taking cancer-fighting dietary supplements, more is better. Whether or not this concept is valid is especially relevant to health-conscious men and women, who are ironically at highest risk for the ill-effects of oversupplementation because they are already consuming high quality diets rich in vitamins and minerals. In 2001, the National Cancer Institute launched SELECT to evaluate whether daily supplementation with selenium (Se) or vitamin E prevents prostate cancer. But very little was known about what dose of Se might offer the most potent cancer-protective effects. We hypothesized that Se regulates the accumulation of genotoxic damage within the prostate and that the relationship is non-linear, i.e. more Se is not better.
Methods: We conducted a randomized feeding trial in which 69 elderly beagles (equivalent to 65 year-old men) received adequate or supranutritional Se intake for 7 months. We used the aging dog prostate to mimic the aging human prostate, enabling us to study the effects of Se on prostatic cells in an appropriate context.
Results: Se supplementation significantly decreased the accumulation of DNA damage in the prostate (alkaline Comet assay). When we examined the relationship between toenail Se level and prostatic DNA damage, we discovered an intriguing U-shaped dose response curve: more was not better. Further, we showed that the Se level that minimizes DNA damage in the aging dog prostate remarkably parallels the Se level that minimizes prostate cancer risk in 2 large human studies.
Conclusions: Now, more than ever, we need a new approach to cancer prevention — personalized cancer prevention (Waters et al, Nutrition and Cancer 2008; 60:1-6). Defining the U-shaped relationship between DNA damage and cancer-modulating nutrients addresses one of the major obstacles to developing personalized cancer-reducing interventions. It follows from this understanding that not all individuals will necessarily benefit from increasing their nutrient intake. Baseline nutrient status should be required for all individuals in prevention trials to avoid oversupplementation.

Early Results of Imatinib as the Active Agent in Control of Aggressive Fibromatosis. An Attempt of Metaanalysis

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Background: Aggressive fibromatosis (AF) is a neoplasm arising from musculoaponeurotic structures with fibroproliferative characteristics. Most patients suffer from the abdominal tumors and other locations. Molecular pathobiology appears to be of clinical value especially when there is a possibility to use targeted therapy with imatinib. This drug can block several molecules to imply clinical benefits in patients whose advanced AF tumors are unresectable and progressed after completion of radiation, and systemic therapy.
Methods: We have described our clinical experience and have searched databases (PubMed, and Medline) to find publications focused on the imatinib therapy for advanced AF. Early results have shown the clinical activity of imatinib as the salvage therapy in patients with advanced AF.
Results: We found only a few publications focused on the role of imatinib as the salvage therapy in advanced AF. The total number of 175 patients with advanced AF were given imatinib at the dose of 400 mg orally daily for more than 2 months. Median treatment time was 9 months. Median time to progression of AF on imatinib was 6.8 months. 13 patients were given 800 mg orally daily when progression of AF on 400 mg of imatinib was recorded. Among of them in 7 patients SD (stabilization disease) was noted. There were following responses to imatinib assessed in 175 patients: 2 CR-1.1% (complete response), 26 PR-14.8% (partial response), 99 SD-56.5%. 127 patients (72.5%) experienced clinical benefits interpreted as disease control. 47 patients had toxicity profile of G1-3 (grade) (no G-4): asthenia-44.6%, nausea and vomiting-40.4%, diarrhoea-25.5%, oedema-25.5%, abdominal pain-12.7%, rash-12.7%. There were also attempts to characterize AF at the molecular level by expression of following KIT-26 positive/44 patients, PDGF receptor alfa-24 positive/44 patients, PDGF receptor beta-31/44 patients, mutations within PDGF receptor alfa exon 12-1case/22 patients, within PDGF receptor alfa exon 18-3 cases/22 patients. Additionally, WNT was mutated in 84% (16 cases/19 patients) but no correlation between mutations and responses to imatinib was noted.
Conclusion: Imatinib is an active drug that is able to be used to control advanced AF remaining unresectable and after completion other therapeutic modalities as radiation, and systemic treatment with anti-inflammatory drugs, endocrine therapy, and chemotherapy.

Antiproliferative Properties of Quinidine

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Background: Quinidine reduces sodium channel activity and is a general potassium channel blocker. While its antiarrhythmic class I properties in curing heart diseases are well known, less information is available about its antiproliferative properties.
Results: It is established that K^+ channel blockers inhibit cell proliferation by leading to membrane depolarization and an arrest in early G1 phase. Recent work demonstrates that quinidine prevents the IGF-1 stimulated cell growth in MCF-7 cells. It interferes with the IGF-1 (Insulin-like growth factor) stimulated activity and expression of hEAG (human ether à go-go) potassium channels through an Akt-phosphorylation dependent pathway. In C6 glioma cells quinidine was found to reduce polyamine levels which are essential for cell proliferation by a block of ODC (ornithine decarboxylase) activity. Further downstream quinidine reduces protein levels of the G1- S transcription factor, E2F1. In addition to its antiproliferative action in breast cancer cell lines or glioma cells lines quinidine was also found to be of potential therapeutic interest for a subset of mesothelioma tumors. While normal Schwann cells remained unaffected in proliferation by quinidine, neurofibromatosis type 2 Schwann cells were down regulated. In addition quinidine was found to be a proliferation modulating agent for human liver cells. Furthermore, quinidine is used as one of the cinchona alkaloids against Plasmodium falciparum growth.
Conclusions: Quinidine has antiproliferative qualities which may be beneficial for a selected number of diseases which are characterized by over stimulated cell proliferation.

Operation as a motivation for smoking cessation

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Background: From the anesthesiologist's view, the hospital stay should be used to aid the patient in smoking cessation, as smoking may lead to prolonged length of stay and numerous cardiopulmonary complications. In addition significantly more general and wound infections that lead to an increased length of stay. In addition the time of the preanesthetic evaluation is perfect as a teachable moment, and should be used for health promoting measures (i.e.: smoking cessation).
Methods: We conducted a survey of 2516 adults in the pre-anaesthesia clinic before surgery. Patients answered questions on a laptop regarding their consumption of alcohol, nicotine and illicit drugs, social and educational background. A total of 1169 patients were asked for the BA.
Results: The percentage of smoker is almost identical in both settings (34.8 % in the PC and 34.5% in BA). The degree of nicotine dependence differs between both settings. The BA revealed 37.2 % of patients with a FTND > 4, while the PC showed 47 %. The share of smokers with an action stage according to the transtheoretical model is much higher in the PC despite the lower nicotine dependence with an average of 27.6 % compared to 4.2 % in the control group.
Conclusions: As in hospital patients have a higher motivation for smoking cessation, the preanesthetic clinic is the ideal place for explicit medical advice and the offer to support in smoking cessation.

Intracellular and organelle-specific drug delivery: „The new frontier for Magic Bullets"

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Sub-cellular, i.e. organelle-specific drug delivery is emerging as the new frontier in drug delivery [1]. It has become more and more evident that the efficiency and efficacy of drug action is dependent largely on how well an unaided drug molecule is able to reach its intracellular target. Subsequently, specific delivery of a drug to its site of action inside cells will dramatically improve its action [2]
Mitochondria play a key role in apoptosis and several clinically used as well as experimental drugs are know to trigger apoptosis by directly interacting with target site at or inside mitochondria. Therefore we hypothesized that that the mitochondria-targeted delivery of such drugs will dramatically increase their pro-apoptotic activity.
We have been developing during the last years a variety of mitochondria-specific pharmaceutical nanocarriers for the purpose of delivering therapeutic DNA or low-molecular weight compounds to mitochondria inside living mammalian cells [3, 4].
Here we summarize our efforts and introduce new data demonstrating the applicability of our mitochondria-targeted nanocarriers for increasing the apoptotic activity of two drugs, paclitaxel [5] and ceramide [6].
Paclitaxel is a molecule that has recently been shown to have pro-apoptotic biological targets on the mitochondria but has a QSAR-predicted cytosolic accumulation and no affinity for mitochondria. Using a mitochondria-specific nanocarrier system (DQAsomes) prepared from the amphiphilic quinolinium derivative dequalinium chloride to deliver paclitaxel to mitochondria in cells we report that it is possible to improve the pro-apoptotic action of paclitaxel. Ceramide is a sphingolipid signaling molecule that has been shown to mediate a diverse range of biological responses to extracellular stimuli. It is well known that mitochondria are the link between the increased levels of ceramide generated by chemotherapeutic drugs and the induction of apoptosis. We report that the specific delivery of ceramide using mitochondriotropic liposomes to the mitochondria elicited a robust apoptotic response while non-targeted delivery failed to do so. In summary, our studies cclearly demonstrated that organelle-specific drug-loaded nanocarriers can significantly enhance therapeutic drug effects.

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Residue Networks and Drug Resistance in Hepatitis C Virus Antiviral Therapy

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Background: Telaprevir (VX-950) and Boceprevir (SCH 503034) are hepatitis C virus (HCV) NS3-4A protease inhibitors with strong antiviral activity in recent phase 1 clinical trials and currently in phase 2 development. We revealed amino acid mutations conferring varying degrees of drug resistance by direct sequencing and clonal analysis for both inhibitors. We apply in-silico approaches and the HCV replicon system to study the molecular mechanisms of drug resistance.
Methods: We use available experimental structures of NS3-4A, together with protein-ligand docking, rotamer analysis and molecular dynamics simulations. Furthermore, we introduce a novel approach using a 2D network formed by the non-covalent interactions between amino acids of the 3D protease structure to interpret the effect of important mutations on replication efficacy and resistance development. An HCV replicon assay was used for IC50 determination of mutant NS3-4A proteases.
Results: We investigate the conformational variability of the protease in alternative ligand-binding models and predict the binding mode of VX-950. We use the 2D network of non-covalent interactions to discover mechanisms of drug resistance in VX-950 and SCH 503034. Mutations at positions 36 and 54 are located spatially close to a hydrophobic cavity in the ligand-binding pocket. We show that the cyclopropyl group in VX-950 is oriented towards this hydrophobic cavity. We also describe the potential impact of mutations at V36 and T54 on the side-chain and backbone conformations and provide possible explanations for their effects on antiviral efficacy and viral fitness.
Conclusions: 1) T54 mutants are expected to interfere with the catalytic triad of the protease and thereby are assumed to affect the viral replication efficacy to a larger degree than V36 mutants. Molecular dynamics simulations of T54A/S mutants and rotamer analysis of V36A/G/L/M side-chains support our interpretations. 2) Mutations at V36 and/or T54 result in impaired interaction with the VX-950 cyclopropyl group, which explain the development of viral breakthrough variants in VX-950 but not in SCH 503034.

Beyond cholesterol lowering: Immunomodulatory effects of lovastatin

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Background: For decades 3-hydroxy-3-methylglutaryl coenzym A (HMG-CoA) reductase inhibitors (statins) such as lovastatin have been used to lower plasma cholesterol levels and in consequence the risk of cardiovascular disease. More recently, there has been an increasing interest in the immunomodulatory effects of statins. These latter effects have been largely attributed to the modulation of the HMG-CoA reductase pathway. Our group discovered an anti-inflammatory mechanism of lovastatin action entirely unrelated to HMG-CoA reductase inhibition.
Methods and Results: We demonstrated that lovastatin selectively blocked the binding of the integrin lymphocyte function-associated antigen-1 (LFA-1) to its ligand intercellular adhesion molecule-1 (ICAM-1). Unexpectedly, lovastatin was found to bind to a hitherto unknown site within LFA-1 distant from the ligand binding site, suggesting an allosteric mode of inhibition (Fig.1). LFA-1 is one of the key adhesion molecules involved in leukocyte migration during an immune response. In agreement with this function lovastatin inhibited leukocyte migration in a murine model of peritonitis and exhibited beneficial effects in murine allergic contact dermatitis. Subsequent optimisation of lovastatin for LFA-1 binding resulted in novel allosteric LFA-1 inhibitors which are under preclinical evaluation for the treatment of autoimmune diseases and transplant rejection.

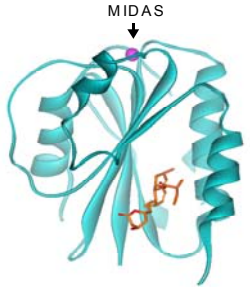

Fig.1: Crystal structure of the complex between the LFA-1 binding domain (I-domain) and lovastatin. The crystal structure shows that the allosteric 'lovastatin site' (L-site) of LFA-1 is located distant from the metal-ion dependent adhesion site (MIDAS) implicated in ligand binding.

Conclusions: Our demonstrate that lovastatin inhibits LFA-1 function by binding to a novel allosteric integrin site. Further investigations are needed to assess whether and to which extent the lovastatin effect on LFA-1 contributes to the overall benefit of the drug in patients.

On the Need for Fractal Modeling of Renal Clearance, *G* (ml/min), of Radiolabled Pentatate

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Background: E_n indicates a Sum of Exponential Terms (SETs) model of n compartments. Using E_1, we prior isolated a normal G predictive formula with exponents at a $P = 0.88$ proximity to $V^{2/3}W^{1/4}$, where V (ml) is volume of distribution and W is mass (kg). This suggested fractal and not compartmental body composition and implied a need for testing SET fits to data.
Methods: Four statistical tests were applied to SET models for studies with 8 or 9 blood samples taken from 10 to 240 min. after injection of ^{169}Yb-DTPA (pentatate, n=41) and 5 to 180 min. after ^{99m}Tc-DTPA (n=5). Test 1 used bootstrap with 1000 resamplings to determine whether the 4 multiplicative and rate constant parameters of an E_2 SET model were all statistically warranted. Test 2 examined residual structure of E_1 and E_2 SET models fit to subselections of samples and classified non-E_2 fit results. Test 3 examined E_2 goodness of fit with Chi-squared and t testing. Test 4 examined extrapolation. For 46 cases, E_2 SET models were fit to m−1 and m blood samples, designated by $E_2(m-1, t)$ and $E_2(m, t)$ respectively, and evaluated and compared at the time of the m^{th} sample.
Results: Test 1 found that E_2 parameter tolerances included 0 for at least 1/4 parameters, 45/46 times using standard error of the mean derived tolerances and 19/46 times using 95% tolerances from 5% range trimming, i.e., the E_2 model was too complex given its performance 41 or 98% of the time, ruling out even more complex $E_{n>2}$, for improving fit performance. In Test 2, the estimated variance of E_1 and E_2 fits varied with the sample times chosen and was worse than that expected for a 3% standard deviation sampling error rate. Upon fitting, 8.1% of the E_2 requests were non-E_2 models. Test 3, goodness of fit testing, found 1) that E_2 fits failed t testing for mean residual magnitude for 6 of 8 residuals of the 41 cases with 8 samples. And, 2) for 46 cases with all samples, the Chi-squared goodness-of-fit was $P<0.039$, suggesting very poor fits. Test 4, extrapolation, showed that $E_2(m-1, t=t_m)$ significantly underestimated $E_2(m, t=t_m)$ (31/46 times, Wilcoxon signed-rank sum $P=0.0035$) thus physical area under the concentration curve over time is underestimated and G, overestimated.
Conclusions: SETs failed testing of model fit to data. Replacement of compartmental models with a fractal consistant model is suggested for fitting DTPA concentration in time.

Magic Bullets: Once Fired – How are They Recaptured? On Metabolic Transformation and Environmental Fate of the Veterinary Fluoroquinolone Enrofloxacin

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Background: Fluoroquinolones (FQs) are synthetic antibiotics widely used to treat bacterial infections in humans and animals. Hence, drug residues enter the environment via urine and feces, i.e., in wastewater streams and animal waste, respectively, the latter often being spread as fertilizer on agricultural fields and pastures. Due to very tight binding of FQs to feces and soils as well as a fluoro-aromatic structure element not yet found in natural products, concerns have been expressed on FQ biodegradability, long-term persistence, and potential selection of FQ-resistant zoonotic species in the environment. Metabolic transformation of enrofloxacin (ENR), a veterinary FQ, is limited to glucuronidation and partial oxidation of its amine substituent. In the only soil degradation study yet published (1997), ≤0.6% of the ^{14}C label applied with [2-^{14}C]sarafloxacin could be recovered as $^{14}CO_2$ after 80 days, prompting our work on the fate of ENR in agricultural matrices.
Methods and Results: In samples of pre-rotted wheat straw, cattle dung pats, a manure hill, and an agricultural soil only about 0.5% of $^{14}CO_2$ was produced from [4-^{14}C]ENR over one year, while $^{14}CO_2$ production rates from [piperazine-2,3-^{14}C]ENR were on the order of 7 to 30%. Similar rates obtained with cipro- and moxifloxacin indicated a limited utility of recording $^{14}CO_2$ formation to prove FQ biodegradation. Quantitative biotransformation of ENR in two agricultural soils, a plant-derived compost, and cattle dung ($t_{1/2}$ = 83 to 113 d) could only be demonstrated, if monitoring of the fate of [4-^{14}C]ENR was based on chemical analysis of the matrices. Degradation pathways, outlined by 135 metabolites, indicated multiple hydroxylations of the aromatic core and the piperazine moiety of ENR, causing oxidative decarboxylation and defluorination as well as the formation of reactive *ortho*-aminophenol- and catechol-type intermediates, which - upon further oxidation - provided labile *cic,cis*-muconic acid-type metabolites (Appl Microbiol Biotechnol 71:90-113; 2006). Seven key metabolites had no or very little residual antibacterial activity. ENR was metabolized by diverse basidiomycetous fungi, indigenous to soils and animal waste.
Conclusions: Soil microbes are likely to provide for the inactivation/recapturing of magic bullets such as ENR, with no risk confirmed.

Transfer factors - Magic Bullets for preventing and treating viruses and mycobacteria in the 21st century

WHITE A

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When the body is exposed to a pathogen, the immune system directs its response down one of two complimentary pathways, denoted as Th2 and Th1. The Th2 response leads to antibody-mediated immunity. That is, immunity that involves the generation of antibodies, which attach to a pathogen floating in the body and disable it. The Th1 response leads to cell-mediated immunity. That is, immunity that involves the identification and destruction of cells in the body that are infected with a virus or mycobacterium. Memories of Th2 immune battles are stored in antibodies and the B-cells that make them. In 1949, immunologist H.S. Lawrence discovered that memories of cell-mediated immune battles are stored in peptides, which he labeled "transfer factors." Like antibodies, transfer factors are passed from mothers to their offspring in colostrum. In the last few decades, researchers have developed protocols for extracting transfer factors from cow colostrum, and also from chicken eggs. By first exposing the cow or chicken to a particular virus or mycobacterium, transfer factors specific to any known pathogen can be generated and extracted. They can be taken orally and produce minimal side effects. Dr. Lawrence labeled them transfer factors because they literally transfer immunity from one host to the next. When taken prior to exposure to a pathogen, transfer factors allow the body to protect itself from infection. When taken after exposure to a pathogen, transfer factors help the body defeat the infection. More than 1000 manuscripts regarding transfer factors have been published in the past 60 years. They represent experimentally verified Magic Bullets in the prevention and treatment of viruses and mycobacteria.

Methotrexate – the past and the present

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Background: Methotrexate (MTX) has a long and an unusual history of use in pharmacotherapy. Moreover, it is characterised by several unique features that are responsible for its popularity as a therapeutic agent: it is active against various cancer and immunologic diseases, including rheumatoid arthritis and psoriasis; it has the broadest dose-range; it can be given by many different routes; it is a cytostatic with a special position in anticancer chemotherapy, as the drug to be recommended for routine determination of its concentration in blood serum and cerebrospinal fluid; it is the drug which has an antidote (leucovorin). Efficient and safe therapy requires broad knowledge of its mechanism of action, pharmacokinetics, toxicity, as well as interaction between simultaneously administered drugs. The therapy with this drug is very effective but its adverse effects are the most common reasons for discontinuing the treatment. Many authors have been trying to find risk factors of its toxicity.
Methods: The studies were carried out in 226 persons: 112 children and teenagers with acute lymphoblastic leukemia (ALL), non Hodgkin`s lymphoma (NHL) and osteogenic sarcoma; 49 adults with rheumatoid arthritis; 65 healthy adults. MTX concentrations in blood serum were determined by the fluorescence polarization immunoassay applying TDx Abbott analyser. The following pharmacokinetic parameters for methotrexate were calculated: elimination rate constant, biological half-life time, apparent clearance, area under the the serum concentration-time curve. Determination of MTX toxicity was based on analysis of biochemical laboratory tests that characterised the function of the liver, the kidney, hematopoietic system and clinical symptoms. Activity of N-acetyl-beta-D-glucosaminidase (NAG), as an early factor of renal tubules efficiency, was determined in urine by the enzymatic method. The influence of diuresis amount on enzyme activity was eliminated by scalling NAG activity in relation to creatinine concentration in urine.
Results: The presented author`s research showed the relationships between MTX concentrations, as well as its pharmacokinetic parameters and biochemical laboratory tests in the children and teenagers with ALL, NHL and osteogenic sarcoma. On the basis of those correlations mathematic equations were formulated for early predicting MTX concentrations, its pharmacokinetic parameters and the values of the biochemical tests connected with the liver and the kidney function. No such correlations was observed in patients with rheumatoid arthritis. In order to predict the drug concentration, the equations allowing the calculation of MTX concentration at 24, 48 and 168 h after administration were formulated using values measured at 1.5 and 24 h after the administration of the drug. In these patients, the mean activity of NAG was significantly increased both before and after treatment with MTX when compared to corresponding values in the control group of healthy subjects.
Conclusions: 1. Determination of MTX concentrations is very important for the optimalization of pharmacological treatment in cancer patients. It could lead to making therapy easier in the patients exposed to toxic effects connected with delayed MTX elimination from the organism. 2. MTX monitoring therapy in patients with rheumatoid arthritis does not significantly improve the effectiveness of the treatment, but it can play an important role in increasing the safety of this drug. 3. The necessity of detailed estimation of kidney excretory function before the beginning and after the end of MTX therapy in the rheumatoid arthritis patients exists. That procedure may be helpful for the recognition of the patients at high nephrotoxicity risk, who need special care.

The Anti-Tumor Action of the Hybrid Drug Nitric Oxide-Donating Aspirin Relies on the 'Passive' Linker and not on Nitric Oxide Nor on Aspirin: An Overview of an Interesting Twist

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Background: Hybrid drugs, by design, rely on the combined action of two pharmacologically active units in one molecule. The hybrid drug nitric oxide-donating aspirin (NO-ASA) consists of aspirin (ASA) and a nitrate group connected through a chemical linker. Its design was based on the assumption that NO, released from the nitrate group, would counterbalance the gastrointestinal side-effect of ASA. Benchmark derivatives are meta-NO-ASA and para-NO-ASA, both having a benzyl-type linker. Both meta-NO-ASA and para-NO-ASA proved to be active anti-tumor agents in preclinical models, generating excitement in the hybrid drug community. Our aim was to study the actual contribution of the nitrate and ASA hybrid components in the most active derivative, para-NO-ASA.
Methods: Structural analysis of NO-ASA led us to synthesize model compounds in which the nitrate- and/or ASA-moiety in para-NO-ASA were replaced by a 'dummy' chloro- and/or acetyl-group, respectively, but with retention of the benzyl-type linker. Organic and computational chemistry as well as spectroscopic analyses were used to study the chemical characteristics of NO-ASA and model compounds. Furthermore, we studied the mode of action of these compounds in colon cancer cells, including the effect on glutathione levels, caspase-3 activation, cyclin D1 expression, DNA fragmentation and cell death.
Results: All the synthesized model compounds, i.e. lacking ASA and/or the NO-donating group, retained or improved upon the in vitro anti-tumor activity of NO-ASA in colon cancer cells. They do so by exhibiting similar (bio)chemical effects as para-NO-ASA does. We show that this is a result of a unified mechanism involving formation of a cytotoxic quinone methide from the 'passive' benzyl linker through ester hydrolysis and subsequent elimination of an intact nitrate ion. This quinone methide depletes intracellular glutathione by a selective chemical reaction. Ultimately, this leads to apoptosis.
Conclusions: Both NO and ASA, paradoxically, are not involved in the in vitro anti-tumor action of para-NO-ASA. Rather, a cytotoxic quinone methide formed from the 'passive' linker is the responsible agent.

Targeted Cytotoxins in Neuroscience: Magic Bullets for Pain Research and Treatment

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Background: Targeted delivery of cytotoxic proteins has been extensively explored as a "Magic Bullet" treatment for cancer with limited success. However, the same approach has proven highly successful in neuroscience, giving birth to "molecular neurosurgery".
Methods: Neuropeptides, such as substance P (SP), dermorphin (mu opiate peptide), neuropeptide Y (NPY) and galanin, can be used to target the ribosome inactivating protein, saporin, to neurons expressing the respective cognate receptors by conjugation through a disulfide bond. The peptide vectors bind to the corresponding receptors and are internalized taking saporin into the target cells. Rats are injected via temporary lumbar intrathecal catheters. After two weeks, the rats undergo nociceptive testing followed by immunohistochemical staining of spinal cord sections for the appropriate peptide receptors to assess loss of target neurons.
Results: Lumbar intrathecal injections of SP-sap, dermorphin-sap, NPY-sap and galanin-sap selectively destroy superficial spinal dorsal horn neurons that express the corresponding receptors but have no effect on dorsal root ganglion neurons. SP-sap in rats transiently reduces hotplate reflex responses, but produces long lasting reduction in operant escape responses to aversive heat and cold and nocifensive responses to hindpaw formalin injection while preventing/reversing hyperalgesia and allodynia due to nerve injury or inflammation. Dermorphin-sap destroys lamina II mu opiate receptor-expressing neurons producing an increase in nocifensive responses to hindpaw formalin and reducing analgesic potency of intrathecal and systemic morphine while NPY-sap and galanin-sap both reduce sensitivity on the hotplate and to formalin in rats. In rats and dogs, SP-sap, at doses that produce significant anti-nociceptive effects, produces little or no evidence of non-specific neural or systemic toxicity.
Conclusions: Molecular neurosurgery with targeted toxins is a powerful research tool in neuroscience and the "Magic Bullets" SP-sap, NPY-sap and galanin-sap have great promise as a new approach to treatment of chronic pain based on targeted ablation of key populations of spinal dorsal horn nociceptive neurons via receptor-mediated endocytosis of the neuropeptide-saporin conjugates.

Red blood cell omega-3 fatty acids and the risk of ventricular arrhythmias in patients with heart failure

WILHELM M

Background: Epidemiological studies support the protective effect of omega-3 fatty acids on sudden cardiac death. However, patients with structural heart disease and an implantable cardioverter defibrillator (ICD) showed no effect or even a proarrhythmic response to fish oil supplementation. Animal studies suggest different electrophysiologic effects of circulating and incorporated omega-3 fatty acids.
Methods: In 102 ICD patients in New York Health Association functional class II or III, the fatty acid composition of red blood cells was analyzed by gas chromatography. The omega-3 index was calculated from eicosapentaenoic acid and docosahexaenoic acid. Patients were followed for 1 year, and ventricular arrhythmias requiring antitachycardic therapy were analyzed. Twenty-five healthy subjects served as control.
Results: In ICD patients, the fatty acid profile was significantly altered and the baseline omega-3 index was significantly elevated, as compared to control subjects (5.12% ± 0.87% vs 4.24% ± 0.96%, P b .001). Kaplan-Meier estimates of probability of ventricular arrhythmias showed significant differences among quartiles of the omega-3 index. Twelve percent of patients in the lowest quartile had ventricular arrhythmias, as compared to 54% of patients in the highest quartile (P = .022). In a multivariate analysis, the omega-3 index was the only independent predictor for ventricular arrhythmias up to 9 months. At 12 months, a reduced ejection fraction was an additional risk predictor.
Conclusions: In heart failure patients, the red blood cell fatty acid profile is altered. Omega-3 fatty acids are elevated and predict the risk of ventricular arrhythmias.

Decoding infections - How to use „administrative" data for clinical quality improvement

WILKE MH

In many countries vast data collections are existing, which all – more or less – are containing coded patient informations. These data collections are mainly used for administrative purposes especially in the DRG (CaseMix) settings they are used for funding, reimbursement, planning, etc. On the other hand the data contain – at least if the respective country is 'mature' in CaseMix – multitudes of clinical and medical information. In some countries even medication information (using ATC-codes) such as France or the U.S. are collected and stored.
Surprisingly enough there are comparatively few publications that are using these data collections to reflect on clinical research questions on a broader basis than e.g. in the own hospital settings. About the reasons can only be speculated, a common allegation – at least among clinical researchers – is that the data quality is not eligible for clinical research as it was collected for 'administrative' purposes.
On the other hand vast data collections are waiting to be exploited and years of workpower for extra double or triple data acquisition for various purposes could be saved.

We started a scientific project "decoding infections" where we developed algorithms and a database that allows us, retrieving information about hospital acquired infections out of the German standard DRG – dataset (§21-dataset) which is a mandatory dataset, that all German hospitals have to provide each year to a central institution called InEK Insitute.

After processing the data of a hospital, we are able to identify – on the basis of the ICD coding – infections that occurred during a hospital stay.

We can analyze clinical outcomes, such as mortality, length of stay, ICU stay, hours of mechanical ventilation as well as economical outcomes such as cost and payments.

Via the database we can show clinicians an overview over infections in their hospital and use the data for discussion about antibiotic therapy strategies, hygiene improvement projects. Moreover we are able to draw the discussion towards the economical impact of hospital infections.

The goal of the project is to provide the data needed to initiate clinical improvement projects on hospital infections and to enable both clinicians and economists in the hospital to measure effects of those projects over time.

In the presentation we'll show the methods and materials as well as some sample results of our work. As the database is still 'under construction' we'll also show our next goals in development such as setting up a benchmarking opportunity for hospitals.

Investigation of Dibenzyl Trisulphide (Dts) Isolated from Petiveria Alliacea as an Immunomodulator with Cytotoxic/Anti- Proliferation Activity

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Background: Cancer is considered to be one of the leading cause of death. The need to find effective and safe therapeutic agents for treating cancers has been one of the greatest challenges for mankind. Thus, 70% of all human cancers such as breast, small cell- lung, malignant melanoma, ovary and colon have remained refractory or untreatable. To date, the most effective drugs known for treating malignant diseases are derived from natural products and their associated derivatives.
Method: The following molecular biological techniques; cell culture, immunostaining, staining of F-actin, SDS-PAGE and Western blotting, tyrosine phosphorylation, proliferation/viability assay and one-dimensional (1D) NMR interaction studies were conducted according to Rosner et al., (2001). Animal studies using mice, were employed for evaluating the cell biological (cytotoxic/anti-proliferation) and toxic effects of dibenzyl trisulphide (DTS) isolated Petiveria alliacea.
Results: Dibenzyl trisulphide increased the weight of the thymus and Peyers patches via cell proliferation. The trisulphide has a dose dependent anti-proliferation effect on the following human cancer cell lines; SH-SY5Y neuroblastoma, MCF-7 mammary carcinoma, IPC-melanoma, A549 small cell lung cancer and 5637 primary bladder carcinoma. The SH-SY5Y neuroblastoma cells were the most susceptible to DTS with an IC_{50} of 0.43 µM. Binding DTS to serum albumin in buffered saline enhanced its cytotoxic effects by 2500 on the neuroblstoma cells. Dibenzyl trisulphide had little or no toxic effects on the non-cancer cell line, HOFA. The mode of action found for DTS is an attenuation of the dephosphorylation of tyrosyl-residues of mitogen activated protein (MAP) kinases (ERK 1 and ERK 2).
Conclusion: The data obtained for DTS suggest that the compound could be an interesting prototype for investigation as an anti-cancer agent.
Reference: Rosner H, Williams LAD, Jung A and Kraus W. (2001). Biochimica et Biophysica Acta (BBA), Molecular Cell Res. Vol. 1540, No. 2, pp. 166 – 177.

Counteracting drug resistance by nutritional management and genetic improvement of disease resistance - lessons from in-silico studies for nematode infections of sheep

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Background: Gastrointestinal parasitism (GIP) is a major challenge to animal production worldwide. Parasite resistance to drugs impedes the control of the disease and raises the need for alternative methods, such as host nutrition and breeding for resistance. Field study results on the efficacy of the two methods are however contradictory and give rise to much speculation. Aims: (1) to explore, in silico, the interactive effect of host genotype and nutrition on performance and GIP and relevant genetic parameters of sheep infected with nematode parasites. (2) to determine under what circumstances nutritional control and / or selective breeding become feasible alternatives to chemoprophylaxis.
Methods: A mechanistic model, describing the growth, nutrient utilization, development of immunity and levels of nematode infections in lambs challenged with GI nematodes was developed. Host genetic variation was assumed in the ability to grow and to resist GI parasites. Simulated breeds differing in growth and resistance genotypes, were infected daily with a trickle challenge of 3000 L3s of Teladorsagia circumcinta nematodes and given access to either good or poor quality grass.
Results: Mean values for production and resistance traits 50 days post infection are shown in the table. Means, heritabilities (fraction of variability due to genetic variation) and correlations for growth and resistance traits changed markedly over time, and were affected more by nutrition than by host genotype. Genetic parameter estimates from field studies were only reproduced when underlying growth and resistance mechanisms were genetically related.

Trait	Good quality feed				Poor quality feed			
	Fast growing		Slow growing		Fast growing		Slow growing	
	Resist.	Suscept.	Resist.	Suscept.	Resist.	Suscept.	Resist.	Suscept.
Body weight	41.4	41.0	35.3	35.0	33.6	33.5	28.3	28.2
Parasite burden	5604	12142	5600	12137	6675	13374	6776	13484

Conclusions: Nutritional management and selective breeding are valuable alternatives to anthelmintics in the control of GIP, but their efficicacy depends on the interaction between host genotype and the nutritional environment.

Rituximab Maintenenance Therapy in CD20+ B-Cell Non-Hodgkin-Lymphoma – First results of a multicenter prospective randomised Phase II study

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Clinical and pharmacokinetic data suggest that the effect of rituximab could be improved by prolonged exposure to the drug. To test for this hypothesis we performed a prospective randomized trial of rituximab maintenance therapy in patients with CD20+ B-cell Non-Hodgkins-Lymphoma. After completion of standard treatment patients were randomized to either observation or maintenance therapy with rituximab (375 mg/m^2) every 3 months for 2 years. Patients after first line therapy as well as relapse patients were included in the study. Patients with aggressive lymphoma were enrolled if they had achieved a complete response (CR) after initial treatment. Patients with aggressive lymphoma with residual tumor mass were examined with positrone emission tomography (PET) and qualified for randomization if PET showed no signs of tumor activity. Patients with indolent lymphoma qualified for the study if at least a partial response (PR) was achieved. After recruitment of 172 patients a planed interim analysis was performed. Complete data sets of 162 patients (pts) with CD20+ B-cell Non-Hodgkins-Lymphoma were evaluable for analysis. Histological subtypes included diffuse large cell lymphoma (69 pts), follicular lymphoma (41 pts), mantle cell lymphoma (18 pts), primary mediastinal lymphoma (15 pts), marginal zone lymphoma (9 pts), Burkitt's lymphoma (3 pts), immunocytoma (2 pts), primary intestinal lymphoma (1 pt), hairy cell leukemia (1 pt), chronic lymphocytic leukemia (1 pt) and unclassified B-cell lymphoma (2 pts). The interim analysis showed that event free survival was significantly prolonged in the rituximab maintenance group compared to the observation group ($p<0.05$). However, no difference in overall survival between the two groups was observed so far. Two patients in the treatment group developed WHO grade III adverse events (1 leucopenia, 1 infection). Both pts recovered shortly after appropriate treatment. We conclude that rituximab maintenance therapy is feasable, safe and well tolerated in patients with CD20+ B-cell Non-Hodgkins-Lymphoma and may prolong event free survival in this patient population.

Reloading Ehrlich's Magic Redox Bullets: Targeting the Redox Achilles Heel of Melanoma Using Phenothiazinium Dyes

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The use of redox dyes as vital stains and metabolic probes was pioneered by Paul Ehrlich, who associated their cytotoxicity with spontaneous autoxidation of the bio-reductively generated leuco-form. Altered redox signaling and regulation in cancer cells represent a chemical vulnerability that can be targeted by selective chemotherapeutic intervention. Here, we demonstrate that 3,7-diaminophenothiazinium-based redoxcyclers (PRC) including methylene blue and toluidine blue O induce selective cancer cell apoptosis by NAD(P)H:quinone oxidoreductase (NQO1)-dependent bioreductive generation of cellular oxidative stress. Using PRC lead compounds against human metastatic G361 melanoma cells, apoptosis occurred with phosphatidylserine-externalization, loss of mitochondrial transmembrane potential, cytochrome C release, caspase-3 activation, and massive ROS production. Consistent with reductive activation and subsequent redoxcycling as the mechanism of PRC cytotoxicity, co-incubation with catalase achieved cell protection, whereas reductive antioxidants enhanced PRC-cytotoxicity. In contrast, human A375 melanoma cells were resistant to PRC-induced apoptosis, and PRC-sensitive G361 cells were protected by preincubation with the NQO1-inhibitor dicoumarol. Indeed, NQO1 specific enzymatic activity was nine fold higher in G361 than in A375 cells. The critical role of NQO1 in PRC-bioactivation and cytotoxicity was confirmed, when NQO1-transfected breast cancer cells stably overexpressing active NQO1 displayed strongly enhanced PRC-sensitivity as compared to vector-control transfected cells with base line NQO1 activity. Based on pilot studies performed in mouse xenograft models and the known overexpression of NQO1 in various tumors including melanoma and lung cancer these findings suggest the feasibility of developing PRC lead compounds into tumor-selective bioreductive chemotherapeutics. Supported in part by grants from NIH (R01CA122484; ES06694) and ABRC (0721).

Applications of recombinant human epidermal growth factor in the treatment of hard-to-heal wounds

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Background: Human epidermal growth factor (hEGF) is a 53 amino acid polypeptide found in our duodenum and salivary glands. It is capable of stimulating cell proliferation and differentiation of various epidermal tissues and has been applied to promote the repair of duodenal ulcer, hepatic injury and eye damage. Our group at the Hong Kong University of Science and Technology has been interested in the production and applications of hEGF in cosmetic and skin care industries. In this presentation, the various skin care applications of hEGF will be discussed.
Methods: Topical application of cream products supplemented with 0.02% (wt/wt) and 0.04% (wt/wt) of recombinant hEGF, which was produced by a proprietary Escherichia coli excretion system[1] (www.gene-vinate.com), onto skin wounds has been reported previously[2].
Results: Our recent study[2] of using hEGF cream products to treat diabetic patients suffering from chronic ulcers revealed that 0.04% (wt/wt) hEGF caused more ulcers to heal over a 12-week period and it was 38% to 53% more effective than 0.02% (wt/wt) hEGF and the negative control, respectively, in healing diabetic foot ulcers. Further studies of the applications of hEGF in the treatment of other hard-to-heal wounds, including drug-induced Steven Johnson syndrome[3], scalded skin, surgical wounds, and psoriasis all resulted in an enhanced healing effect. The results support our view that topical application of hEGF, when administered at an effective dosage, may offer a simple and effective treatment to the management of various skin wounds.
Conclusions: 1) Topical application of hEGF-containing cream to skin wounds helps enhance the healing effect and reduce the healing time. 2) Topical application of hEGF presents a simple and effective treatment to the management of a broad range of skin wounds.
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Feasibility of Mapping Brain pH Using 31P MR Spectroscopy

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Background: Magnetic resonance (MR) spectroscopy is a valuable method for the noninvasive investigation of metabolic processes. Although brain adenosinetriphosphate (ATP) studies can be found in multi-voxel ^{31}P MR spectroscopy, previous studies of intracellular brain "potential of hydrogen" pH was conducted in single-voxel ^{31}P MR spectroscopy. Aims: To explore the feasibility of mapping brain ATP and brain pH by using multivoxel ^{31}P MR spectroscopy.
Methods: Phantom studies were carried out by using a GE 3T scanner firstly. Many available sequences were tested using phantom and the two dimensional (2D) Point RESolved Spectroscopy Chemical Shift Imaging (PRESSCSI) sequence was selected because of better signal to noise ratio. Time of repetition (TR) was 1000 msec and time of echo (TE) 144 msec with 128 scan averages. The acquisition matrix was 16 x 16 phase encodings over a 24-cm field of view (FOV). Slice thickness was 10 mm. Then a healthy volunteer from MR research team was studied. Data were processed offline using the Spectroscopic Analysis of General Electric / Interface Definition Language (SAGE/IDL) software. Baseline and phase corrections were performed. Multivoxel spectra and brain ATP map were analyzed. Brain pH values were calculated from the difference in chemical shifts between inorganic phosphate (Pi) and phosphocreatine (PCr) resonances. Color scaling map was generated using MatLab software.
Results: Multivoxel ^{31}P spectra were obtained for phantom and the healthy volunteer. PCr map was obtained in phantom. At this moment, peaks of PCr were not homogeneous in phantom studies. There was noise for multivoxel ^{31}P spectra in volunteer study. Phosphomonoester (PME) peak, P*i* peak, phosphodiester (PDE) peak, PCr peak, γATP peak, αATP peak, and βATP peak can be identified. Preliminary brain ATP map and brain pH map were generated in the volunteer.
Conclusions: It is feasible to map brain ATP and brain pH using multivoxel ^{31}P MR spectroscopy. However, endeavors should be made to improve quality of multivoxel ^{31}P MR spectroscopy.

The G-rich promoter and G-rich coding sequence of basic fibroblast growth factor are the targets of thalidomide in glioma

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Background: Despite the very high risk of teratogenicity, thalidomide is emerging as a treatment for cancer and inflammatory diseases. Thalidomide is considered to be an effective drug for treating refractory multiple myeloma due to its antiangiogenic and immunomodulatory activities. In addition to myelomas, thalidomide has been widely tested on various types of tumors, such as renal cell carcinoma, prostate cancer, glioma, and Kaposi's sarcoma. Clinical efficacy in some inflammatory conditions, including graft-versus-host disease after allogeneic bone marrow transplantation and renal transplantation, further supports the immunomodulatory properties of thalidomide. Although current data provide much promise for the use of thalidomide in the treatment of these diseases, its mechanism of action is still incompletely understood. Earlier clinical studies have found that patients responding to this drug often had high plasma levels of basic fibroblast growth factor (bFGF). This cytokine is a proangiogenic factor overexpressed in many tumors and is also a regulator of limb development; hence, it might be a target of thalidomide. bFGF belongs to the FGF gene family and is a potent
autocrine and paracrine mitogen that is ubiquitously expressed. Secretion of bFGF is independent of the traditional ER-Golgi pathways. Besides the secreted form, which is translated using the first AUG codon, there exist four nuclear target forms of bFGF. These four forms are translated differently from upstream in-frame CUG codons through an internal ribosome entry site (IRES)-dependent mechanism. Due to the different intracellular distribution and the NH2-terminal extension of high molecular weight (HMW) bFGFs, the functions of these HMW bFGFs compared with the low molecular weight (LMW) bFGFs are believed to be different. bFGF is overexpressed in various types of tumors, such as glioma and renal cancer, and increased expression of bFGF has been found to be correlated with disease progression. The expression of bFGF transcripts is under the control of a G-rich promoter. In addition to transcriptional regulation by the G-rich promoter, the NH2-terminal–extended bFGF coding sequence is also G-rich, which may function to regulate translation of different isoforms. This RNA region may also serve as a target for some DNA-binding drugs and consequently modulate expression of the isoforms. Drucker et al. reported that thalidomide at relative high concentrations (>25 μg/mL) could downregulate transcription for genes with GC-rich promoters.Additionally, Stephens et al. proposed that thalidomide may inhibit insulin-like growth factor–induced and bFGF-induced limb genesis because both genes were under the control of GC-rich promoters. The transcriptional and the translational regulation of bFGF are under the control of G-rich–containing sequences, which can interact with thalidomide. Considering that bFGF is a ubiquitous growth factor involved in many biological activities, we hypothesized that the G-rich sequences of bFGF are the major targets of thalidomide. The present study was to determinate the possible molecular mechanism of thalidomide as well as to highlight the feasibility of using low-dose thalidomide to treat tumors as glioma in a clinical setting.
Methods: To examine the antitumor effect of thalidomide, we used U-87 MG cells, which are a high-grade human glioblastoma cell line expressing high basal levels of bFGF Real-time reverse transcription-PCR analysis was used to assess the mRNA levels of bFGF in U-87 MG cells with and without thalidomide treatment. Because the 5′-end of the bFGF transcript is GC-rich, we postulated that translation of bFGFmay also be affected by thalidomide. Western blot analysis was therefore done to analyze the levels of the various bFGF isoforms. Because bFGF has been shown to promote cell transformation, a soft agar colony formation assay and hanging drop technique were used to assess the effects of thalidomide on anchorage-independent and three-dimensional growth abilities of U-87 MG cells, respectively. we next examined whether the tumorigenicity of these cells was reduced by down-regulation of bFGF expression. Three different bFGF shRNAs (#1, #2, and #3) or control shRNA was introduced into U-87 MG cells by lentivirus infection to generate three bFGF knockdown clones. To evaluate the effect of thalidomide on transcription driven by the bFGF promoter, a pbFGF-EGFP plasmid, containing a portion of the bFGF promoter to drive the expression of EGFP, was stably transfected in U-87 MG cells to give U-87-bFGF-EGFP cells. Cells were treated with thalidomide (0.1–10 μg/mL) for different time intervals, and then transcript levels and relative fluorescence indices were measured to evaluate the effect of thalidomide on the expression of EGFP. Because downregulation of the HMW bFGFs by thalidomide was more significant than LMW bFGF, we asked whether IRESdependent translation of bFGF, which was reported to regulate the expression of different isoforms, was also affected by this drug. HMW and LMW bFGF IRES fragments were inserted into bicistronic vectors to generate pHMW-IRES and pLMW-IRES plasmids. Both plasmids were then stably transfected into U-87 MG cells to give U-87-HMW-IRES cells and U-87-LMW-IRES cells. After treating with liposome encapsulated thalidomide for 12 h, IRES activity was estimated by calculating the ratio between firefly luciferase activity and Renilla luciferase activity. The former represented the translational efficiency of the upstream cistron, and the latter represented that of the individual bFGF IRES. To examine whether thalidomide interacted preferentially with the G-rich coding sequence of bFGF, we measured the UV-VIS absorbance of thalidomide after incubation with a G-rich (nucleotides 363–428) or a non–G-rich (nucleotides 673–768) DNA fragment derived from bFGF. We hypothesized that the absorbance of thalidomide may be diminished more dramatically by the secondary structure of a DNA fragment that was bound more tightly by this drug.
Results: Using U-87 MG cell lines, we found that thalidomide, especially when encapsulated in a liposome, down-regulated the transcription and translation of the FGF-2 gene by interacting with G-rich regions present in the promoter and the internal ribosome entry site of its transcript at concentrations much lower than therapeutic serum concentrations. Thalidomide treatment also dramatically suppressed the anchorage-independent growth of U-87 MG and other glioma cells by over a thousand fold without affecting its anchorage-dependent growth, which may be accomplished by knocking down endogenous bFGF expression in these cells. Accordingly, the addition of recombinant bFGF partially restored the anchorage independent
growth of these cells. In addition, the absorbance at 230 nm was quenched to a greater extent on thalidomide treatment when it was incubated with a G-rich DNA fragment, suggesting that this drug may bind preferentially with nucleic acids that have a high content of guanosine.Our data suggest that by targeting the G-rich regions of bFGF, thalidomide (at 0.1 μg/mL) can reduce cellular bFGF levels and affect tumor anchorage-independent growth, the hallmark of tumorigenicity.Our results are promising for future clinical investigations using low doses of thalidomide.

Authors' disclosure statement **(not counting towards the character count):**

Low-dose thalidomide : novel mechanism & new insight in cancer therapy
Although the mechanism of action responsible for the effects of thalidomide remains unclear, this drug is currently under investigation for the treatment of several disease types, ranging from inflammatory conditions to cancer. Using liposome encapsulation for preventing the rapid hydrolysis of thalidomide, Mei and Wu found that low concentration of thalidomide as 0.1μg/ml target the G rich region of bFGF in glioma thereby diminishing cellular bFGF levels and suppressing anchorage- independent tumor growth. The data may have important implications for understanding the mechanistic rationale for low-dose thalidomide use in the clinical realm of cancer therapy.

Two vaccines too far: the poliovaccine fiasco of 1935

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Background: Animal models are useful – but may be misleading. The Kolmer and Park-Brodie vaccines were based on inadequate basic knowledge. It was assumed that both vaccines contained live poliovirus. A more subtle explanation does not exclude this possibility, although the virus was probably non-infectious by the route used and did not cause the few paralyses and deaths. As well as virus, the vaccines contained monkey spinal cord tissue which causes inflammatory reaction when injected into humans. This inflammation would have mimicked provocation. Other polioviruses (of low virulence) were circulating and the coincident inflammation would have increased their apparent virulence in a few vaccinees and caused the few cases of polio.
There had been good evidence for the theory of genetic susceptibility to polio, but the vaccine fiasco seemed to suggest that a safe vaccine was not possible. The theory was forgotten. Instead it was thought that while small doses of virus gave immunity, large doses resulted in polio and that paralytic cases excreted large quantities of virus (resulting in family cases) – neither of these theories could be tested.
Conclusions: However plausible, theories unsupported by evidence can be wrong. Later research has shown that there are two groups that are genetically susceptible. Ehrlich's pioneering work of monitoring vaccine use was forgotten until the 1950's.

Disturbance in energy metabolism induced by hepatotoxins in a liver spheroid model

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The liver plays a central role in the metabolism and transformation of energy-generating substances. Mitochondria and different metabolic pathways in hepatocytes interact and are often vulnerable targets of toxicants. Using animal tests it is difficult to evaluate liver energy metabolic functions accurately because energy-generating substances are also taken up or released by other cells in the body; it is also difficult to achieve high-throughput. We have developed a rat primary liver 3D spheroid cell model which retains metabolic functions found in vivo. Based on this model, a test called the 'spheroid cell spreading inhibition test' (SCSIT) was used for determining suitable exposure concentration ranges for test compounds. The effects of the hepatotoxins diclofenac, isoniazid, paracetamol and galactosamine on the metabolism of energy-generating substances (glucose, pyruvate, lactate and galactose) by liver spheroids were evaluated. The results show that all the toxicants tested significantly reduced glucose and lactate release ($p < 0.01$). Diclofenac, isoniazid and paracetamol significantly reduced pyruvate uptake ($p < 0.01$), whereas, galactosamine did not affect pyruvate uptake within the concentration range tested. Diclofenac, galactosamine and paracetamol significantly decreased galactose uptake ($p < 0.01$); by contrast, isoniazid did not show a significant adverse effect. It is concluded that the primary liver spheroid model can mimic in vivo liver function in terms of the metabolism of energy-generating substances. The combined use of several endpoints of energy metabolism can result in reliable toxicity evaluation. High drug concentrations can kill cells rapidly, and may not therefore generate useful functional information on toxicity. As a method for predetermining toxicant concentration ranges, the SCSIT is therefore a reliable, useful and necessary tool for studying the effects of toxicants on liver functions and will have applications for drug safety screening and high throughput testing.

Biomedical Research at The University of The West of England, Bristol

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The Centre for Research in biomedicine (CRIB) is a key functional research grouping of international repute that brings together expertise in genomics, cell signaling, inflammation, molecular biology, gene regulation and cell biology to promote research and knowledge exchange in critical areas of biomedicine. Research teams within the Centre support over 20 post-doctoral researchers and more than 30 post-graduate research students.
The primary aim of CRIB is to understand fundamental cellular and molecular biomedical processes and identify potential markers and targets that can be developed into novel diagnostic and therapeutic strategies. The Centre is not only engaged in finding new insights into the understanding of human disease but has a strong focus in the translation of the knowledge into new health policy, diagnostics and therapeutics.
Current projects include: Non-invasive prenatal diagnosis, genetic blood typing, endotoxin and biomarkers for sepsis, insulin vesicle mobilization and diabetes, molecular microbiology and bioluminescent bacteria, 3D cell culture models and in vitro toxicology, volatile organic chemical analyses for clinical diagnoses.

Targeted Immunotherapy of Cancer Through TCR Gene Transfer

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Background: Conventional cancer therapies are limited by their toxicity and lack of specificity. To achieve targeted immunotherapy, we have targeted Wilm's Tumour antigen (WT1) which is overexpressed in most leukaemias and many solid cancers, and isolated WT1-specific T cell receptor (WT1-TCR) genes. Human T cells transduced with WT1-TCR eliminated leukaemia cell lines *in vitro* and in a NOD/SCID mouse model. To facilitate the clinical application of TCR gene therapy, we have modified this WT1-TCR retroviral construct aimed at improving TCR functional activity and reducing its cytotoxicity.
Methods: WT1-TCR constructs were generated in a retroviral vector in which the woodchuck hepatitis virus-derived post-transcriptional regulatory element was deleted. To enhance desired pairing, a second disulphide bond was introduced between the TCR α and β chains and the human TCR constant domains were replaced with murine sequences. Following transduction, the functional activity of WT1-TCR engineered T cells was tested by ^{51}Cr release cytotoxic T lymphocyte (CTL) assay and intracellular cytokine staining. After the engraftment of $CD34^+$ leukaemia progenitor cells from a CML patient in NOD/SCID mice, adoptive immunotherapy was performed with WT1-TCR engineered patient's T cells. Animal survival and leukaemia burden was monitored.
Results: We have generated WT1-TCR constructs with improved safety features and enhanced functional activities as determined by tetramer staining, CTL killing and intracellular cytokine staining. To mimic the clinical settings, we engrafted $CD34^+$ leukaemia progenitor cells into NOD/SCID mice, followed by adoptive immunotherapy with the patients' T cells transduced with either WT1-TCR or control TCR. We show that mice treated with WT1-TCR engineered CTL had a greater survival than the mice treated with control CTL. Analysis of the bone marrow showed that control mice had engraftment of leukaemia cells, while WT1 CTL treated mice did not. These data provide a solid basis for a phase I clinical trial.
Conclusions: 1). Genetic modification of TCR genes provides a way of generating safe, efficient reagents for clinical applications. **2).** WT1-TCR transduction of patient's T cells offers a simple and efficient way of producing tumour specific T cells for the treatment of leukaemias. This has important implications for treating other WT1-expressing cancers.

A cost-effective Haemophilus Influenzae type b conjugate vaccine in combination with DTwP-Hep B vaccine for developing countries – A perspective

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Background: Despite the availability of Hib conjugate vaccines, Hib remains a leading cause of meningitis and pneumonia deaths, worldwide. Advent of combination vaccines has greatly simplified immunization activities and also helped in additional vaccines being incorporated into immunization schedule. IAP reviewed data on combination vaccines and declared that addition of Hib/Hepatitis B vaccines to DTwPw vaccine as safe and effective for primary as well as booster vaccination. This study was conducted to assess the immunogenicity and reactogenicity of DTPw HB + Haemophilus Influenzae type b conjugate vaccine manufactured by SIIL in comparison with Tritanrix HB + Hiberix vaccine of Glaxo Smith Kline (GSK) in Indian Children aged 6-14 weeks.
Methods: 304 children aged 6-8 weeks at enrollment were equally randomized to receive three doses of 0.5 ml of either SIIL or GSK vaccine intramuscularly, with a gap of one month between each dose. Pre and Post vaccination IgG antibody titres were assessed by ELISA at an independent laboratory using Good Laboratory Practice (GLP) guidelines. Reactogenicity was assessed from the frequency of adverse events recorded by parents on diary cards. Study was conducted as per International Conference on Harmonization - Good Clinical Practice (ICH-GCP) guidelines and Declaration of Helsinki.
Results: Demographic characteristics were comparable in both the groups. Post-vaccination GMTs in SIIL group were 2.78 IU/ml, 50.87 U/ml, 1.34 IU/ml, 616.73 mIU/ml and 7.55 µg/ml for anti Tetanus, anti-Pertussis, anti Diphtheria, Anti HBs and anti-PRP. Corresponding values in GSK group were 2.52 IU/ml, 48.28 U/ml, 0.99 IU/ml, 463.12 mIU/ml and 7.82 µg/ml respectively. Post-vaccination sero-protection was 100% for all components, in both groups except Pertussis component, which was 96.06% in SIIL group and 95.4% in GSK group. Frequency of adverse events was higher with the first dose and thereafter reduced in both the groups. Common adverse events were pain (SIIL-42%, GSK-44%), redness (SIIL-15%,GSK-14%), swelling (SIIL-29%,GSK-30%) and fever (SIIL-41%, GSK-45%). No Serious Adverse Event was reported in the study.
Conclusion: 1) DTPw HB + Hib vaccine manufactured by Serum Institute of India Ltd., Pune was found to be safe, immunogenic and non-inferior to GSK vaccine 2) This vaccine is also cost effective and therefore affordable to a larger population in the developing world.

In Vivo Analysis of Brain Muscarinic Receptor (mAChR) Occupancy by Anticholinergic Agents Analyzed via Quantitative Autoradiography (ARG) and Positron Emission Tomography (PET)

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Background: For anticholinergic therapy for overactive bladder (OAB), it is concerned that chronic administration of these agents in older patients may result in a non-degenerative mild cognitive impairment, depending on the ability to occupy brain mAChR. Aims: 1) To characterize in vivo brain mAChR occupancy by anticholinergic agents for OAB treatment.
Methods: At 10 min after iv injection of oxybutynin (Oxy), solifenacin, tolterodine or darifenacin in rats, [^{11}C](+)3-MPB (selective mAChR radioligand) was injected (iv), followed by removal of brain for ARG. Following oral Oxy, rhesus monkeys were injected (iv) [^{11}C](+)3-MPB, and PET scan was performed. mAChR occupancy was estimated by distribution volume of each brain region. Plasma concentrations of Oxy and its active metabolite, N-desethyloxybutynin (DEOB) were measured by LC/MS/MS.
Results: In ARG study, there was a dose-dependent decrease in in vivo specific [^{11}C](+)3-MPB binding in rat brain regions after iv injection of anticholinergic agents, indicating brain mAChR occupancy. Dose ratios of anticholinergic agents for brain mAChR occupancy (RO_{50}) to inhibition of carbachol-induced increase in intavesical pressure (ID_{50}), which reflects in vivo bladder selectivity to brain, were significantly greater for solifenacin and tolterodine than Oxy. Darifenacin displayed little occupancy of brain mAChR. In PET study, following oral administration of Oxy (0.1, 0.3 mg/kg) in conscious rhesus monkey, plasma concentrations of Oxy and DEOB were dose-dependently increased, with greater concentration of DEOB. At 1-4 hr after oral Oxy at these doses, mAChR occupancy in brain regions was 40-60%. The plasma concentrations in rhesus monkey after oral Oxy administration were similar to those in humans received clinical dose of Oxy.
Conclusions: Oxy occupies brain mAChR under in vivo condition, suggesting a risk of CNS adverse effect under clinical condition. In the treatment of OAB, CNS side effects could be avoided by newer generation of anticholinergic agents with high bladder selectivity. In conclusion, in vivo ARG and PET analysis of brain mAChR occupancy may provide fundamental basis for managing CNS side effects in anticholinergic therapy for OAB.

Noninvasively Evaluation on Human Stress Using Salivary Biomarker

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Background: Saliva sampling has the advantage that it is noninvasive, making multiple sampling easy and stress free. Salivary amylase activity (SAA) can be a useful index of plasma norepinephrine concentration under a variety of stressful conditions, since it appears that increased sympathetic nervous activity is a major stimulator of amylase secretion. In order to realize a hand-held monitor of the sympathetic nervous system, we fabricated a completely automated analytical system for SAA using a dry-chemistry system.
Methods: The monitor consisted of a disposable test-strip and an optical analyser (130 × 87 × 40 mm^3; 190 g), which was incorporated within an automatic saliva transfer device. The test-strip consisted of a collecting paper and a reagent paper containing 2-chloro-4-nitrophenyl-4-O-β-D-galactopyranosylmaltoside (Gal-G2-CNP), a substrate for amylase. The collecting paper is directly inserted into an oral cavity, and approximately 30 µl of whole saliva is collected from under the tongue. When Gal-G2-CNP is hydrolyzed by amylase, the hydrolyzed product (CNP) develops a yellow color and the reflectance is measured by an optical device. The definition of one unit activity (U) per mass of enzyme is that this activity produces 1 µmol of the reducing sugar, maltose, in 1 min. The SAA by video viewing was observed in 64 healthy adults. The ethical committee approved the study.
Results: When this monitor was used, it took 30 s for saliva sampling, 30 s for saliva transfer and measurement, and a total of one minute was enough to measure the SAA. The calibration curve of the monitor was within a range between 10 and 140 kU/l showing a coefficient with R^2 = 0.97. With regard to reproducibility of the measured results for the saliva transfer volume of the same samples, the coefficient variation (*CV*) was 5.5%. In our time course experiment, the SAA level was increased just after the beginning of stressful video viewing in 63 of 64 subjects, and immediately returned to the pre-sress level just after the end of the video viewing. On the other hand, the SAA level was significantly decreased by smoothing video viewing.
Conclusions: It was demonstrated that the manufactured monitor enabled a user to automatically measure the SAA with a high accuracy. The SAA measurement will be powerful tool for psychological research.

Soluble Leishmanial Antigen and Plasmid Expressing Interleukin-12 Protects BALB/c Mice from Leishmania major Infection

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Background: In murine leishmaniasis, the induction of the T-helper type 1 (Th1) response contributes to infection resistance, whereas the establishment of the Th2 response makes the mice susceptible to infection. Interleukin-12 (IL-12) plays a pivotal role in the diversification of immune responses to the Th1 type. In this study, we tested whether the co-administration of IL-12 expression plasmid and leishmanial antigen will skew highly susceptible BALB/c mice to Th1 response and protect from leishmaniasis.
Methods: The BALB/c mice were intradermally injected with the combination of IL-12 plasmid which compose p35 and p40 subunits and soluble leishmanial antigen (SLA) 7 days prior to the challenge with lethal dose (1x10^6) of promastigotes of Leishmania major. The courses of disease and immune responses of the mice were assayed.
Results: The mice which received IL-12 expressing plasmid plus SLA completely healed and the parasite burden in the local lymph nodes significantly decreased. The cured mice attained long-term immunity, and were resistant to any subsequent rechallenge of the lethal dose of the parasite. The protective effect was associated with the development of a Th1 response, as demonstrated by the enhanced level of antigen-specific interferon-□ (IFN-□) and dominant production of IgG2a in the serum. In contrast, the administration of empty plasmid plus SLA or IL-12 plasmid alone failed to protect the disease and shape the Th1 response. Furthermore, the protective efficiency induced by the vaccination was clearly prevented by the injection of either neutralizing anti-IL-12 mAb or anti-IFN-□ mAb.
Conclusion: The IL-12 expression plasmid is thus an effective adjuvant for the elicitation of a protective Th1 response against leishmaniasis and is therefore, considered to be appropriate for vaccinations that require the induction of Th1 type immunity.

Antitumor Activity of New Combination Chemotherapy with Iirinotecan Hydrochloride(CPT-11) and Nedaplatin(NDP) against Human Cervical Cancer Cell Lines

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Background: Antitumor activity of combination chemotherapy with irinotecan hydrochloride(CPT-11) and nedaplatin was compared to that with CPT-11 and cisplatin.
Methods: In vitro cytotoxicity of SN-38 (an active metabolite of CPT-11) in combination with nedaplatin or cisplatin was evaluated using three human cervical cancer cell lines(ME-180,CaSki and SiHa).Interactions between two drugs in combination were investigated using a simultaneous-exposure schedule and analyzed by the IC50-based isobologram method. In vivo antitumor effects of CPT-11 in the combination with each platinum were studied using SiHa xenografts.
Results: IC50 values of nedaplatin against these three human cervical cancer cell lines were about 2-fold as high as those of cisplatin, indicating somewhat weak cytotoxic effects of nedaplatin. Simultaneous exposure to SN-38 with each platinum preparation showed synergistic and additive effects against ME-180 and SiHa. While CPT-11, nedaplatin and cisplatin alone hardly showed any antitumor effects even at the maximum tolerated dose (MTD) levels, the combination chemotherapy with CPT-11 and nedaplatin or cisplatin resulted in significant antitumor effects even at three-quarter MTD of CPT-11 combined with two-third MTD of platinum. All treatments were tolerable for mice, indicating that the combinations did not cause significant enhancement in toxicity.
Conclusions: In clinical application, nedaplatin causes a lower incidence of nephropathy and does not require the replacement of a large volume of fluid, which is needed for cisplatin administration, facilitating treatment at the outpatient clinic. In addition, the incidences of digestive disorder, peripheral neuropathy and auditory disorder are lower.These findings suggest that the combination chemotherapy with CPT-11 and nedaplatin for squamous cell cancer of uterine cervix is very useful in clinical practice. A dose-finding study should be conducted.

Clinical trial of immunochemotherapy (personalized peptide vaccination and gemcitabine) for metastatic pancreatic cancer patients

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Background: The present multicenter phase I/II study was conducted to confirm the efficacy and toxicity of immunochemotherapy for metastatic pancreatic cancer.
Methods: HLA-A2 or A24 positive patients with histologically or cytologically proven pancreatic adenocarcinoma with at least one measurable metastatic lesion were eligible for the study. Other eligibility criteria included: age of 20-80 years, ECOG performance status (PS) of 0 or 1, and adequate organ function. Gemcitabine was given intravenously at a dose of 1,000 mg/m^2 over 30 min once a week for three weeks, followed by 1 week of rest, and three or four peptides that had been positive for pre-existing peptide-specific immune responses in the circulation (personalized peptide vaccine) were injected subcutaneously in the femoral area once a week. The objective response rate was assessed according to RECIST.
Results: Twenty patients from 2 institutions were enrolled in this study. The median age was 63 years (range: 41-80 years). The PS was 0 in 10 patients (50%) and 1 in 10 patients (50%). The efficacy and toxicity were analyzed in 20 patients who received at least one course (8 weeks) of immunochemotherapy. Although no complete response was seen, a partial response was achieved in 5 patients, resulting in an overall response rate of 25%. Eleven patients (55%) had stable disease. The median overall survival was 9.0 months with a 1-year survival rate of 33%. The major grade 3-4 toxicities using CTCAE criteria were neutropenia (25%), anemia (20%), thrombocytopenia (20%) and anorexia (5%). All 17 dermatologic reactions at the vaccination site were scored as grade 1 or 2. No treatment-related deaths occurred during the study. Augmentation of peptide-specific CTL responses in the post-vaccination peripheral blood mononuclear cells was observed in 75%, while increased titer of peptide-specific IgG antibodies was observed in the post-vaccination plasma in 80%. Conclusions: This study could be recommended for further stages of clinical trials because of safety, boosting of immune responses, and potential clinical benefits.

Interfere IGF-I pathways to augment sensitivities of colorectal cancer cells to current therapeutic agents

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Current chemotherapy agents are not highly effective against disseminated colorectal cancers. One major contributor to the limited effectiveness of the treatment is cancer cell producing high level of IGF-I and IGF receptors which has been proven protecting a broad range of colon cancer cells from a variety of apoptotic challenges caused by chemotherapy agents. The IGF system, therefore, has become an attractive molecular target for anticancer therapies. Based on IGF-I structure we have designed and synthesised a novel IGF type I receptor antagonist. The effect of the antagonist on human colon cancer cell proliferation was examined by a non-radioactive assay; the apoptosis was revealed by determining the activities of cellular caspases 3/7 8, 9. The apoptosis pathways were investigated by examining the level of pro-apoptosis singling protein with Western blotting. The results showed that IGF-I receptor antagonist induce cancer cell apoptosis and inhibits colon cancer cell proliferation. The changes of Caspase 3/7, 8 and 9 activities and different expression level of Bax in cancer cells after treatment with the peptide suggested that the extrinsic pathway may play an important role in IGF-I receptor antagonist induced apoptosis in colon cancer cells. Treating different type of colorectal cancer cells by combination of IGF-I receptor antagonists with current chemotherapeutic agents has shown that IGF-I receptor antagonists significantly augment sensitivities of colorectal cancer cells to current chemotherapeutic agents. All these data suggested that IGF-I receptor antagonists could be developed to a therapeutic strategy for colorectal cancer.

Immunogenicity of mucosally delivered Lactococcus lactis expressing a malaria protein in rabbits

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Oro-nasal immunization is preferable to injections from the point of view of safety, ease of administration and compliance. The gram positive food grade bacterium Lactococcus lactis is a potential vehicle for delivering immunogens to the mucosal immune system. We have investigated the immunogenicity in rabbits of the Plasmodium falciparum merozoite surface protein MSA-2 expressed on L. lactis. The 819 bp coding sequence of Plasmodium falciparum MSA-2 was presented on L. lactis in two forms viz. as an intracellular molecule and one covalently anchored to the peptidoglycan layer of the cell wall. The recombinant L. lactis were delivered intranasally or orally for mucosal immunization. Intramuscular immunization was also used as a control. Serum antibody response was investigated by immunofluorescnce assay. High titre serum antibodies that recognize native parasite MSA-2 in immunofluorescence assay were generated on oral and intramuscular immunization. The surface displayed form was more immunogenic than intracellular MSA-2. Either the surface or intracellular form of MSA-2 did not elicit high titres on intranasal immunization. Recombinant L. lactis is a suitably safe vector for subunit vaccines. The strain expressing cell wall anchored MSA-2 elicited higher levels of serum antibodies than strain expressing MSA-2 in the cytoplasm with all three routes of immunization. Systemic IgG antibodies could be generated through mucosal immunisation.

Atorvastatin protects spinal motor neurons from glutamate mediated neurotoxicity

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In the recent few years several studies have demonstrated that statins,in addition to their lipid-lowering effects,have neuroprotective properties. These properties of statins have beneficial effects in neurological disorders. We have analyzed the atorvastatin in a postnatal organotypic spinal cord cultures exposed to glutamate. Cultures were treated for two weeks. Cultures treated with glutamate alone had significant reduction of motor neuron numbers. Cultures treated with glutamate and atorvastatin showed sparing the organotypic morphological appearance. This results showed the atorvastatin plays an important role in the survival and maintenance of spinal motor neurons in their neuroprotection against glutamate induced neurotoxicity. This indicates a potential therapeutic use of atorvastatin in treating diseases that kill motor neurons,such as motor neuropathy and motor neuron disease.

Membrane transport of alkaloids and anticancer drugs in plants: specificity and analogy to mammalian cells

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Background: Higher plants produce a large number of secondary metabolites, which are classified as alkaloids, terpenoids, phenols, and quinones. Some of them, e.g. plant alkaloids, are utilized as medicines for the purpose of anticancer, antibacterial, analgesic, and so on. They are often accumulated in particular sink organs where they are biosynthesized, while some of them are translocated from source cells to sink organs via long distance transport.
Methods: We have been characterizng the function of plant transporters in heterologous system, e.g. Xenopus oocyte, budding yeast, and insect cells depending on the compatibility of the expressin of those membrane proteins. We also used transgenic tobacco to characterize the human ATP-binding cassette (ABC) transporter, MRP1.
Results: We identified an ABC transporter CjMDR1 as a primary transporter for an isoquinoline alkaloid berberine in Coptis japonica, in which this transporter molecule mediates the unloading of the alkaloid at the plasma membrane of the rhizome for its accumulation. In contrast, the vacuolar sequestration of berberine in this plant is mediated by a proton gradient-dependent transport mechanism at the tonoplast.
In the comparison of behavior of human MRP1, we characterized the membrane localization and transport function in tobacco cells. Surprisingly human MRP1 was localised to vacuolar membrane in tobacco cells, while it could recognize daunorubicin as the substrate. By contrast, etoposide did not seem to be transported by the MRP1 when expressed in tobacco cells. This is probably due to the difference in conjugation systems between plant and mammalian cells, i.e. etoposide is transported as glucuronate in mammalian cells, while glucuronide does not play an important role in plant for the detoxification of drugs.
Conclusions: 1) Isoquinoline alkaloid, berberine, is effluxed by P-glycoprotein (ABCB1) in mammalian system, while it is transported in an inward direction in Coptis japonica by an ABCB-type transporter. 2) For the efficient detoxification of the endogenous alkaloid, the berberine-producing cells sequestrate this toxic compound by proton-antiporter at the vacuolar membrane. 3) When expressed in plant, mammalian ABC transporter may show different transport ability due to the altered membrane localization and the different detoxification counterpart in the conjucation reaction.

Vaccines: The Magic Wand of Indian Public Health?

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Backgroud: Adoption of vaccines as magic bullets in disease prevention is not the same as treating them as the magic wand of public health. In a country like India, where the government bears the entire cost of universal childhood vaccination for 25 million newborns every year, vaccination decisions have to be based on economic prudence and evidence-based policy. As disease patterns keep changing, vaccination decisions must be based on disease surveillance, pathogen variation, incidence levels that qualify for selective vs. universal use, logistics and cost-benefit analysis.
Aims: To analyse the changing Indian vaccine policies and practices with reference to 1) the roles of public and private sectors 2) adoption of new vaccines 3) Individual vs. Combination vaccines 4) National priorities and Internationalpressures.
Methods: Historical, economic and policy analyses based on various official documents, literature and field surveys.
Results: Over the last two decades, Indian vaccine scenario has witnessed: 1) a drift away from the policy objectives of self-reliance; 2) declining role of the government and public sector and increasing role of the private sector in production and policy; 3) widening demand-supply gaps in primary vaccines due to private sector's emphasis on new and expensive vaccines 4) adoption/promotion of new vaccines without adequate epidemiological proof of their need and cost-benefit analyses, 4) private sector using combination vaccines (combining universal and non-universal vaccines) to gain backdoor entry into universal vaccine markets, 5) emphasis on vaccine 'coverage' rather than on the 'protection' achieved, 6) overall vaccine strategies drifting along "supply push" rather than "demand pull" arguments and 7) Ignorance or complacency in local government and lack of suitable policy support from international organizations to reverse these trends.
Conclusion: 1) Indian vaccine policies and practices are increasingly being driven by the market forces rather than evidence-based decisions, leading to shortage of affordable primary vaccines, proliferation of unnecessary new vaccines or their expensive combinations. 2) Immunization policies must be based on scientific principles and not by market vagaries. 3) The success of vaccination must be determined by protection achieved and not by money spent or 'coverage'.

New Anti-Endotoxins Agents against Gram-Negative Bacterial Infections

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Background: Endotoxins (lipopolysaccharides, LPSs) are the major components of the outer membrane of Gram-negative bacteria that at higher endotoxin levels cause toxic effects and a number nonspecific patothysiology changes of organism. The present work reveals an interaction of LPS with natural polysaccharides – chitosan and carrageenans – and opportunity of application of polysaccharides as preparations for treatment of gram-negative the infections connected to accumulation endotoxins in organism.
Methods. FTIR, absorbance-, fluorescent-, photon correlation - spectroscopy, microscopy, ultracentrifugation, ELISA, Human embryonal kidney cells transfected with TLR4, patomorphological researchers of bodies and biochemical analyses of blood of mouse, clinical effects of polysaccharides in therapy of 120 patients with food toxic infection on the basis of infectious hospital.
Results. LPS interact with the chitosan and produce stable complexes. The interaction is a complicated process and depends of various factors. The conversion of ultra-structure of LPS by action of carrageenans was observed. Acute toxicity of LPS decreases 20-fold and 5-fold in mixture with chitosan and carrageenan, respectively. An activity of the induction of TNF by LPS with polysaccharide was 70% lower than that of the parent LPS. Chitosan-induced secretion of the pro-inflammatory cytokines in macrophages is not dependent on TLR4- receptors. The activation of cells by carrageenan occurs through specific for LPS TLR4 receptors. Chitosan and carrageenans increase non-specific resistance to impact of LPS-induced endotoxinemia in mice. Polysaccharides hampered the involution of thymus, the changes on level of thyroid hormones in serum, the activation of glycogenolysis, peroxidation of lipids in liver. Carrageenan restored system of a hemostatic and corrected parameters of immune system of organism in the course of treatment of patients with food toxic infection of Salmonnela etiology.
Conclusion: 1. Marine polysaccharides have the highest affinity to endotoxins and modulate significantly the biological activity of LPS. 2. Chitosan and carrageenan can be useful as preparations of auxiliary therapy in clinical practice at gram-negative infection.

ATP and adenosine as biomarkers for drug development

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Background: ATP and adenosine are major regulators of energy metabolism maintaining optimum balance between oxygen supply and demand within the cardiovascular system. In addition to these fundamental roles, they are also potential biomarkers for cardiovascular protection, and as therapeutic targets for treatment of ischemic heart disease (IHD), stroke, inflammatory diseases, cancer, and neurodegenerative diseases. The effectiveness of many cardiovascular drugs could be attributed to their effect on increasing plasma and tissue concentrations of adenosine and ATP. The main objective of our research effort in this program is to determine whether or not adenosine and ATP concentrations in plasma and RBC are useful surrogate biomarkers for development of cardiovascular agents in vivo.
Methods: Male New Zealand White rabbits (4 – 6 kg), normotensive SD rats and hypertensive SHR (325 – 400 g) were used for these investigations. They were treated with a known cardiovascular drug or normal saline (control), and kept either in a restrainer or placed on a research treadmill for an exercise test for blood sample collection and hemodynamic recordings. Blood samples were collected via an indwelling catheter from a carotid artery and immediately mixed with a stopping solution to stabilize adenosine and ATP during subsequent processing. Plasma concentrations of adenosine and red blood cell (RBC) concentrations of ATP and their metabolites were measured by a previously validated HPLC. Data between groups were compared by ANOVA and differences considered significant when $p < 0.05$.
Results: We have found that many cardiovascular agents block the uptake of adenosine by RBC, and that some of them enhance the hemodynamic effects of adenosine in the rabbit model. In addition, we have found that SHR have higher plasma concentrations of adenosine and RBC concentrations of ATP than the normotensive SD rats, and that exercise increases the concentrations of these biochemical regulators particularly in rats treated with the cardiovascular agents.
Conclusion: Many cardiovascular drugs and perhaps also drugs affecting energy metabolism could modulate the circulatory concentrations and cardiovascular effects of Adenosine and ATP which could be exploited for development of novel drugs and innovative therapies. (Supported in part by a grant-in-aid from CIHR/NSHRF/PEF Regional Partnership Program).

Antineoplastic effects of an aurora B kinase inhibitor against breast cancer

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Aurora B kinase is an important mitotic kinase involved in chromosome segregation and cytokinesis. It is overexpressed in some human breast cancer as well as other cancers, and has been linked to genetic instability, tumorigenesis and invasive disease. Aurora B is an important molecular target for chemotherapeutics, and several small molecule inhibitors of Aurora B are now in clinical trials.
AZD1152 is a dihydrogen phosphate prodrug which is converted in the serum to AZD1152-HQPA [hydroxyquinazoline pyrazol anilide]. AZD1152-HQPA is a small molecule ATP binding pocket competitor that selectively inhibits Aurora B [Ki = 0.36 nM] compared with Aurora A [Ki =1369 nM] and 50 other kinases. AZD1152 has been shown to have antineoplastic activity in acute myelogenous leukemia, multiple myeloma and colorectal cancer. To date, AZD1152 has not been evaluated in human breast cancer, the leading cancer among US women.
We investigated the effect of AZD1152 on breast cancer cells. AZD1152-HQPA inhibited a panel of human cell lines including Her18, a Her-2 overexpressing breast cancer line. The IC50 for these lines were 14-125 nM and in the same range as reported by others. Time-lapse photomicrography of Her18 cells treated with AZD1152-HQPA showed enlarged multinucleate cells. Micronuclei and chromosome bridges were observed. FACS analysis demonstrated polyploidy consistent with the increase in DNA copy number in multinucleate cells. AZD1152-HQPA treatment resulted in decrease in Aurora B activity (as indicated by decrease in phosphorylated Histone H3), mitotic failure and apoptosis (as indicated by Annexin V-FITC FACS, increases in PARP cleavage and increase in Bax). AZD1152-HQPA inhibited the clonogenic potential of Her18 cells. AZD1152 (62.5 mg/kg/day on days 1 and 2 of a 7-day repeating cycles) also demonstrated significant antineoplastic activity against Her18 xenografts in nude mice. Reduction in phospho histone H3 was demonstrated by immunoblotting in tumor samples from the AZD1152-treated group. Bax was increased in the AZD1152-treated tumors. Immunohistochemistry for Ki-67 and activated caspase 3 showed that AZD1152 reduced proliferation and increased apoptosis in the treated xenografts.
In conclusion, these data suggest that AZD1152 may be an effective chemotherapy agent for human breast cancer. Further investigation and possibly a clinical trial in breast cancer patients are warranted

Fluoroquinolones in the Treatment of Tuberculosis: Current Status and Future Issues

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In the 1980s to 1990s, fluoroquinolones were shown to have significant activities, both in vitro and in the murine model, against Mycobacterium tuberculosis. Later on, the clinical activity of fluoroquinolones in treating both drug-resistant and drug-susceptible tuberculosis, was demonstrated in some largely uncontrolled chemotherapy trials. The utility in the former setting became rather important due to the surge in multidrug-resistant tuberculosis globally in the past two decades. In parallel, fluoroquinolones were also found to have a useful role in treating patients with significant intolerance to conventional antituberculosis drugs. Furthermore, in order to improve patient adherence and curtail development of drug resistance, a genuine need arose to discover more efficacious drug regimens to shorten the six-month duration of standard antituberculosis chemotherapy for drug-susceptible disease. This prompted focused research on the sterilizing capacity of fluoroquinolones against M. tuberculosis persisters in disease lesions, especially regarding that of the newer 8-methoxyquinolones. Animal experiments have apparently demonstrated the potential ability of these new agents in shortening the duration of therapy, in comparison to standard short-course treatment, and producing stable cure of disease without relapse. Controlled clinical studies are now ongoing to evaluate these issues in patients with tuberculosis. Some preliminary results appear rather promising. However, the emergence of resistance against fluoroquinolones in M. tuberculosis strains, often in the deadly form of extensively drug-resistant tuberculosis would pose a great concern regarding the effectiveness of these new agents in treatment of the disease. Novel strategies are thus required to circumvent this challenge.

Pharmacokinetic and Drug Metabolism: A Bioanalytical Perspective

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Drug development is a complex process that requires technical and scientific expertise in many area and contributive specialist knowledge integration. Evaluation of the pharmacokinetics (PK), drug metabolism, and pharmacodynamics (PD) is essential for understanding the characteristics of a new molecule entity and is necessary for a rational drug development program. Among these processes a key component is the development of bioanalytical methods needed to accurately quantify biological samples of administered drugs. This is an important factor in the study of the relationship of dose, exposure and effect, and reaching reasonable PK/PD conclusions regarding the drug safety and efficacy. A fundamental flaw in drug development program would be the lack of reliable effectiveness and safety profiles and rich database to support pivotal dose regimen design for the clinical trials. Validated bioanalytical assays ensure accurate information regarding PK properties and further the establishment of informative doseexposure (PK)-response (PD) relationship throughout the drug development program, and eventually provide the best possible foundation relevant to more efficacious and safer therapeutic use of the drug in man.

Inhibitory balance of γ-aminobutyric acid in cerebellar circuitry in autism: a circuit-centered approach to drug target design for developmental neurobiological disorders

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The maintenance of the balance between excitation and inhibition in the brain is essential to avoid pathological consequences. γ-aminobutyric acid (GABA) is the principal source of inhibition, it uses glutamate (the principal excitatory transmitter) to block over-excitation in the synapses of the brain and thus preventing seizures. GABAergic agents that enhance inhibition in the brain are anticonvulsive and anxiolytic. A dilemma arises during brain development when a mismatch between the strength of GABA and glutamatergic synapses which may prevent growth and synapse formation and perturb the sculpting of neuronal circuit connections, If this condition prevails, there will be a failure of neuronal communication and may contribute to disordered information processing in autism. Therapeutic drug design based on drug-receptor interaction becomes more challenging in disorders of developmental neurobiological origin involving multiple signalling processes such as autism. The discovery of drug development program in autism will require the application of basic science research with therapeutic modalities that can address malfunctions in the homeostatic mechanisms of the disorder. A circuit-approach study of inhibitory neuromodulation, via post mortem brain studies from autistic compared to control individuals, represents one of our efforts to identify key neurosubstrates in autism as a prelude to future rational drug design.

GAD67 mRNA decrease in cerebellar Purkinje cells in autism: an in situ hybridization study

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Reduced levels of the GABA-synthesizing enzyme, glutamic acid decarboxylase (GAD) isoforms have been reported by Fatemi et al (2002; Soc Biol Psych, 52: 805-10) in the cerebellum of adult autistics but the specific cerebellar regions or cell types were not identified. A likely candidate is Purkinje cells in the posterolateral cerebellar hemisphere, an area previously identified as having moderate to severe decrease of Purkinje cell number. To investigate the role of GAD in Purkinje cells, the current study measures GAD67 mRNA levels in the posterolateral cerebellar cortex.
Fresh frozen brains for the Harvard Brain Tissue Resource Center and Autism Tissue Program consisted of 8 autistics, and 8 controls matched for age, PMI, gender and pH. In situ hybridization procedure was described in Nielsen and Soghomonian (2004; Neurosci, 123: 31-42). Each section covered with 2-8 ng of radiolabeled cRNA was hybridized for 3.5 hr at 50°C. Brain sections radiolabeld with ^{35}S-GAD67 probe were processed for film radioautograph. Area of occupancy of reduced silver grains (μm2) corresponding to GAD67 mRNA in individual neurons was quantified on emulsion radioautographs using NIH image software. GAD67 mRNA levels were reduced by 40% ($p = 0.002$, student t-test) in autistic compared to control (mean ± SEM for controls 1.54 ± 0.08, autistics 0.92 ± 0.09) brains, suggesting downregulation of GAD67 mRNA in Purkinje cells in autism. Whereas GAD67 mRNA levels were reduced, the effects were not paralleled by a significant loss of Purkinje cells (mean ± SEM for controls 4.0 ± 0.2, autistics 3.7 ± 0.2). The reduction in GAD67 mRNA level may result in reduced GABA input to deep cerebellar nuclei disrupting cerebellar output. This may also contribute to autism neuropathology seen in the cerebellar nuclei such as previously demonstrated by Bauman and Kemper (1985; Neurol, 35: 866-74).

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The fecundity ofSchistosoma japonicum was impaired by administration of low dose cyclophosphamide

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Background: Development of female schistosomes from infectious cercariae to mature egg producing adult is the key pathogenic step of schistosome infection. To explore the factors promoting the development of schistosome will helpful for developing some new methods of anti-pathological process. Aim: To explore the impact of administrating low-dose cyclophosphamide (CTX) on the level of CD4+CD25+ Treg cells of mice and on the fecundity development and egg production of S. japonicum.
Methods: This study included 72 C57BL/6J female mice, which were divided into control group, single treating group and consecutive treating group (24 mice per group, weight: 20±3g). The mice in single treating group were given a single low dose of CTX (50mg/Kg) by I.P., mice of consecutive treating group were given a dose CTX per week by I.P., and mice of control group were given placebo of normal salt solution (NS). Mice were infected with 30±1 cercaria of S. japonicum at seventh day after first injection. The level of CD4+CD25+ Treg cell in peripheral blood and spleen of three mice from each group were examined by FACS per week. All the remained mice were sacrificed at 42th day post infection□and the worms recovered from portal vein by perfusion and eggs in liver were counted. The size of egg granuloma was checked by histological observation. This experiment repeated twice.
Results: The percentage of CD4+CD25+ Treg cell in peripheral blood and spleen of mouse was declined and reached to the lowest level at seventh day after received single dose CTX, then rose gradually. The percentage of CD4+CD25+ Treg cell of mice received CTX treatment consecutively maintained a low level constantly. The egg reduction rate was 31.2 to 36.2 % in single treating group, and was 46.1 to 50.4 % in consecutive treating group. The egg granuloma size was reduced significantly both in single treating group and in consecutive treating group than the one in control group, the reduction rate of average equivalent area of single granuloma was 51.5% in consecutive treating group and 19.2% in single treating group respectively.
Conclusion: CD4+CD25+ Treg cell play an important role during the fecundity development of S. japonicum.

Metalloporphyrn-Loaded Liposomes as Anticancer Drug Delivery System. Novel Drug Bearing SOD-like and Fenton-reaction Activities

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Background: A novel design of anticancer drug delivery system of ironporphyrin-loaded liposome (FeP/liposome) is reported. A lack of cytotoxicity of FeP/liposome and an efficient generation of toxic OH· from $O_2^{\cdot-}$ through the iron-catalyzed dismutation and the Fenton reaction allow for targeted necrosis of tumor cells where the $O_2^{\cdot-}$ concentration is locally increased as a result of reduced activity of SOD and catalase in the cells.
Methods: Lewis lung carcinoma tumor cells were treated with some drugs (0 to 100 □M). Cytotoxicity was determined by staining the cells with Alamar blue to obtain the effective concentration (EC_{50}) of drug required to produce 50% lethal dose against cell. Rate constant (k_{cat}) of iron-catalyzed $O_2^{\cdot-}$ dismutation was analyzed by stopped-flow method. Particle size (□) of liposome was measured by DLS and TEM. The relative hydrogen peroxide resistance against SOD (H_2O_2 resist) of FeP was determined by UV-vis.
Results: Significant damage to such impregnated tumor cells is observed in comparison with cisplatin and mitmycin c. The lack of cytotoxicity is shown in control experiments using SOD and manganese-porphyrin (MnP) in place of FeP, which suggests that the cell damage is induced by OH, considering that SOD and MnP only show $O_2^{\cdot-}$ dismutation but do not participate in Fenton reaction. The k_{cat} of FeP/liposome is analogous to that of SOD, which also indicates that has a potential to act as catalyst of $O_2^{\cdot-}$ dismutation. The FeP/liposome is porphyrin-doped nanoparticle and has a strong H_2O_2-resistance. The properties of the liposome will permit its continuous circulation in blood, porphyrin-based tumor targeting, and accumulation in a pathological site such as tumor tissue.

drugs	EC_{50} (□M)	k_{cat} ($M^{-1}s^{-1}$)	□(nm)	H_2O_2 resist
FeP/pH-sens liposome	6.5-7.0	$1.2\text{-}3.1\times10^7$	33-54	280
FeP/liposome	13-14	$1.0\text{-}3.1\times10^7$	23-60	350
cisplatin	31-35	-	-	-
mitomycin c	26	-	-	-
SOD	> 100	2.3×10^9	-	1

Conclusions: We have demonstrated that FeP/liposome bearing SOD-like and Fenton reaction activities present a potential target for design and development of novel anticancer drug. Our approach opens the door for liposome-loading and tumor-targeting of a much expanded selection of metalloporphyrin.

Incorporation of Paclitaxel into Well-Defined Amphiphilic Block Copolymer Micelle Having Phospholipid Polymer Sequence

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Background: Paclitaxel (PTX) is one of the most effective and commonly used drug for the treatment of various cancers. Due to the poor solubility of PTX in water and in many other acceptable pharmaceutical solvents, a specific solubilizing agent such as Cremophor EL is used to formulate PTX in a commercial injection solution. However, serious side effects have been reported. Therefore, a much safer solubilizing reagent has been needed. To realize safer and effective drug administration, novel well-defined and biocompatible micelles of amphiphilic diblock copolymers containing Phospholipid polymer sequences were synthesized.
Methods: At first, the homopolymer of 2-methacryloyloxyethylphosphorylcholine (MPC) was synthesized in water by reversible addition-fragmentation chain transfer (RAFT) controlled radical polymerization. Using this MPC homopolymer, AB type diblock copolymers of n-butyl methacrylate (BMA) were synthesized. Association behaviors of the amphiphilic diblock copolymer ($pMPC_m$-$pBMA_n$) with varying pBMA block lengths were investigated by nuclear magnetic resonance (NMR), fluorescence probe, static light scattering and quasi-elastic light scattering measurements. A given amount of PTX was dissolved in ethanol, and the PTX solution was added to an aqueous solution containing various amounts of the block copolymers. The solubility was evaluated by the transparency of the solution.
Results: Fluorescence spectra of the probe indicated that the probe was solubilized in polymer micelles in water. The formation of polymer micelles comprising a core with pBMA blocks and shell with hydrophilic pMPC block was confirmed by NMR and light scattering data. The size and mass of the micelle increased with increasing pBMA block length. PTX dissolved well in aqueous solutions of the block copolymer as compared with pure water, implying that PTX is incorporated into the hydrophobic core of the polymer micelles.
Conclusions: Well-defined amphiphilic block copolymers composed of pMPC and pBMA blocks were prepared via RAFT controlled radical polymerization. The amphiphilic block copolymer formed polymer micelles in aqueous solutions because of hydrophobic associations among pBMA block. By use of the diblock copolymer, the water solubility of PTX can be improved.

Methotrexate in Dermatology through 50 Years

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Background: Historical evaluation. Methotrexate (MTX) is a cytostatic, well established in treatment of malignancies. It is the oldest of drugs used today for systemic therapy of psoriasis. 1958 was the start of MTX in dermatology. Originally it was prescribed daily for 5-10 days followed by a rest period of a few days. Since 1970 weekly oral or parental doses became the established way and together came trials of combination therapy. In 1978 it was found that MTX lead to more than 75% improvement in 90% of psoriatic patients.
Methods: Studies of mechanisms of action and establishment of side-effects. Despite many years use of MTX, there are uncertainties concerning mechanisms of action. Among the many are cytokine inhibition (Interleukin 1 and 6, and tumor necrosis factor (TNF) alpha) and inhibition of neutrophil leukotriene and platelet activity factor (PAF). Besides inhibition of adhesion, immune-modulation, and aspects of anti-inflammatory properties, a main effect is induction of apoptosis in activated T-cells. In mid nineteen-seventies it was well established, that long-term usage of MTX could induce liver damage, which in some cases led to fibrosis or even cirrhosis. Decline in popularity of MTX followed due to a "liver scare", as it did due to the recommendations that liver biopsies be performed to monitor treatment. We do not recommend a pre-treatment liver biopsy unless one is dealing with a high risk factor for liver disease. Measuring aminoterminal propeptide of type III procollagen (PIIINP) is preferable. This is a non-invasive marker of fibrogenesis, that can show that as long as PIIINP is normal, no significant development of fibrosis is taking place. Dermatologists are also in increasing numbers using MTX in diseases like dermatomyositis, cutaneous sarcoidosis, scleroderma, pemphigoid and pemphigus, Behcet's disease, impetigo herpetiformis and mycosis fungoides. This will often be given in combination therapy as in psoriasis. Among the latter can be mentioned combinations of MTX with the TNF-alpha inhibitors etanercept or infliximab, where a large number of patients over several years have benefited.
Results and Conclusion: If the rules for accepted control are adhered to, the risk/benefit ratio for MTX should be considered one of the most valuable treatments in dermatology. The biologics are generally, but not always of higher dimension in relation to clearing.

New View of Retroviruses; Consequences in Aids Diagnostics and Therapy

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Despite unquestionable success in diagnostics and therapy of AIDS, it is still not possible to stop the worldwide pervasion of this infection. This situation is challenging for us to fight against this disease more complexly and overcome all taboos and dogmas (HIV=AIDS) established in diagnostics and therapy of this disease. The fundamental, still unanswered question is the origin of the HIV. The dogma, which originated HIV by transferring from African apes/monkeys to human population some decades ago, doesn't explain tremendous increase of AIDS incidence, mainly in Asia.
Our untraditional approach is based on original detection of the HIV-like sequences in intestinal bacteria of American and Slovak HIV/AIDS patients by colony and dot blot hybridization assay. Using specific PCR primers for gag, pol and env HIV-1 genes, were synthesized PCR products on bacterial template, which were found in more than 90% homologous to the corresponding HIV-1 sequences. The hight capacity of intestinal bacteria isolated from HIV-positive patients to enter into HL-60 cells and normal human lymphocytes was confirmed by gentamicine protection assay. The reduction of bacteria bearing HIV-like sequences was performed by per oral application of probiotics bacteria Escherichia coli strain Nissle 1917 to AIDS patients. After three months of probiotics treatment, the remission of the viral load was found in 61% of tested patients.
The achieved results allowed us to: 1. predicate that in the AIDS process intestinal bacteria play a basic or even the key role; 2. create a new hypothesis dealing the induction of immunodeficiency with new therapeutic possibilities.

Structural and Functional Diversity of Endogenous Antimicrobial Oligopeptides

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Background: Chemical substances named as oligopeptides consist of 2 to 50 amino acid residues. Natural oligopeptides may regulate nearly all vital processes. To date, primary structures of more than 7000 oligopeptides have been identified. Aims: 1) To elucidate all known natural antimicrobial oligopeptides; 2) To describe their structural-functional diversity; 3) To point their usage in medicine, veterinary, and food conservation.
Methods: This study was performed using our EROP-Moscow (Endogenous **R**egulatory OligoPeptides) database (http://erop.inbi.ras.ru/). It contains complete information on natural oligopeptides.
Results: It have been shown that chemical structures of natural oligopeptides have been identified from more than 1000 different species representing all the biological kingdoms. More than 1500 oligopeptides possess antimicrobial functions. They are active against bacteria, fungi, viruses, stimulate antiviral and antitumoral resistance. These substances were found out in animals (1211), bacteria (109), fungi (162), plants (162), and viruses (2). It is known 121 human oligopeptide structures. Primary structures of antimicrobial oligopeptides are characterized as having widely diverse sequences. Many of them display a net positive charge, ranging from +2 to +18. Nearly all antimicrobial oligopeptides form amphipathic structures upon interaction with target membranes and exhibit antimicrobial activity against a wide variety of micro-organisms. Antimicrobial oligopeptide structure and function offers hope for discovery and development of improved agents to prevent or treat infectious diseases caused by pathogens that resist conventional antimicrobial agents. Various human oligopeptides show a potent effect on pathogenic micro-organisms including antibiotic-resistant bacteria. Moreover, human defensins indicate that these oligopeptides are involved in various biological processes associated primarily with defensive and regulatory responses to infections by pathological agents.
Conclusions: Mammalian antimicrobial oligopeptides without any dangerous side effects due to their natural origin 1) can be used in therapy and veterinary; 2) can be considered as natural preservatives of food products. 3) are promising for hygiene and cosmetics.

Portal Haemodynamics and Plasma Transaminase Levels before and after Prostacyclin Analog Administration in Patients with Chronic Viral Hepatitis

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Background: Under physiological conditions, hepatic stellate cells produce prostacyclin to regulate the haemodynamics of portal microcirculation; in chronic liver disease, there is a progressive dysfunction of hepatic stellate cells and a reduction in prostacyclin production. The administration of a prostacyclin analog might optimize the regulation of hepatic stellate cell function in patients with chronic liver disease. Aims: 1) To evaluate the hepatic haemodynamics and 2) to monitor the inflammatory indexes before and after a prostacyclin analog infusion. **Methods:** 11 patients (6 males and 5 females; mean age 60.7±8.7 years) with histological diagnosis of chronic viral hepatitis were enrolled in the study after the informed consent document was signed by each-one. In each patient, before and after 3 days of physiological solution infusion (placebo) and before and after 3 days of prostacyclin analog infusion (at a dosage of 2 ng/Kg/min for 6 hours/day), serum levels of liver enzymes (GOT, GPT) were evaluated and, with color Doppler sonography, portal flow velocity (cm/s) and portal diameter (cm) were measured to calculate portal flow volume (PFV) and congestion index (CI).
Results: After prostacyclin analog infusion, patients had an increase in PFV ($p<0.01$) and a decrease in CI and GOT serum levels ($p=0.01$ and $p=0.05$, respectively), as compared with pre-prostacyclin analog infusion. No statistically significant difference was observed in GPT serum levels.
Conclusions: After 3 days of prostacyclin analog infusion, a significant amelioration of the hepatic haemodynamics (as demonstrated by the increase in PFV and the decrease in CI) and of the liver inflammatory indexes (as demonstrated by the decrease in GOT serum levels) was achieved. 1) All patients had a better portal liver perfusion. 2) 5 patients had an excellent decrease in liver enzymes while in only one patient their levels worsened. 3) The mean GOT transaminase serum levels were significantly lower relative to those of pre-prostacyclin analog infusion; 4) the liver function seems to benefit from prostacyclin analog infusion, but prospective studies on a larger population are needed.

Neuropeptides in Stress-Related Disorders: Possible Interaction of Vasopressin and Corticotropin Releasing Hormone

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Background: Despite the great importance the treatment of affective disorders is not solved, yet. New drugs target neuropeptides, especially vasopressin (AVP) and corticotropin releasing hormone (CRH) as important regulatory molecules of the stress axis. Aims: 1) Summarize the available data on the role of AVP and CRH in affective disorders based upon their stress axis regulation. 2) Discuss the possible interaction of the two molecules.
Methods: This study will present our own data from AVP-deficient Brattleboro rats in scope of the literature. Behavioral studies (anxiety on elevated plus maze, depression-like behavior in the forced swim test etc.) are combined with analysis of the stress axis by plasma hormone content measurements (radioimmunoassay) and molecular biological methods (e.g. in situ hybridization).
Results: AVP-deficient animals are less anxious and show lower prevalence of depressive-like behavior in behavioral tests. These behavioral changes do not go parallel with changes in hormone levels. The CRH mRNA levels in the nucleus paraventricularis hypothalami are higher in AVP-deficient rats. Our results are supported by studies on high anxiety rats as well as by human data. The contribution of V1a or V1b receptors seems to be controversial. Moreover, several studies suggest that CRH rather than AVP is the key molecule of the process.
Conclusions: 1) AVP seems to be causally involved in the development of anxiety and depression. 2) The participating brain AVPerg circuits are distinct from those regulating the stress axis (different receptor subtypes should be targetted), however their activations may go parallel. 3) Complex intervention on both AVP and CRH system may lead to better results in therapy.

Nasal Vaccination against Bacterial Toxins

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Background: Toxins produced by infectious bacteria often cause fatal diseases. Vaccines against bacterial toxins have been demonstrated as effective countermeasures against infectious diseases. However, with traditional immunizations by needle injection, there remain concerns about the availability of medical service and social acceptability. Our research aims to develop highly efficient and easily administered nasal vaccines against bacterial toxins.
Methods: This study tested a prototypical nasal vaccine against botulinum neurotoxin type C (BoNT/C). A replication-incompetent adenoviral vector encoding a human codon-optimized heavy chain C-fragment (H_C50) of BoNT/C was produced in the laboratory. It was evaluated as a mucosal vaccine against BoNT/C in a mouse model. Groups of mice were intranasally administered with single doses of adenoviral vector Ad/opt-BoNT/C-H_C50, ranging from 10^5 to 2×10^7 plaque forming units (pfu). Serum and mucosal Anti-H_C50 antibody responses were measured by ELISA. The neutralization capacity of anti-sera from mice vaccinated with Ad/opt-BoNT/C-H_C50 was determined by *in vitro* toxin neutralization assay. The protective efficacy of the nasal vaccine was assessed by challenge with up to $10^4 \times MLD_{50}$ of active BoNT/C.
Results: Single intranasal inoculation of the Ad vector elicited a high level of H_C50-specific IgG, IgG1, and IgG2a in sera and IgA in mucosal secretions as early as 2 weeks after vaccination. The antigen-specific serum antibodies were maintained at a high level at least until the 27th week. Immune sera showed high potency in neutralizing BoNT/C, as indicated by *in vitro* toxin neutralization assay. The mice that received a single dose of 2×10^7 pfu of Ad vector were completely protected against challenge with up to $10^4 \times MLD_{50}$ of BoNT/C. The protective immunity showed vaccine dose-dependence from 10^5 to 2×10^7 pfu of adenoviral vector. In addition, animals that received a single intranasal dose of 2×10^7 pfu Ad vector could be protected against $100 \times MLD_{50}$ 27 weeks after vaccination. Animals with preexisting immunity to adenoviral vector could also be vaccinated intranasally and protected against lethal challenge with BoNT/C.
Conclusions: These results suggest that the adenoviral vector is a highly effective gene-based mucosal vaccine against BoNT/C. This study has set up a platform to develop needle-free nasal vaccines against bacterial toxins.

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New Development of Drug Solubilization

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Background: Approximately one of every two new drug candidates identified by high-throughput methods suffers from a very low water solubility, which usually gives rise to poor or erratic bioavailability, and consequently these compounds are frequently not taken forward for further development. Therefore, water-solubilization of poorly soluble drugs is a considerable challenge for drug discovery and development. The commercially available polyoxyethylene-based nonionic surfactants, such as Cremophor EL and Span 80, are used as drug solubilizers, but their applications are limited by their relatively low solubilizing capacity and serious side effects. Amides in conjunction with a hydrophobic chain were examined here because 1) amides should have greater solubilizing power than alcohols, ethers, etc. 2) amides are enzymatically hydrolyzable (yet chemically stable); and 3) amides, being both hydrogen-bond acceptors and donors, may improve the solubilization capacity by interacting with drug substances having hydrogen-bonding sites.
Methods: Twelve amide-based surfactants ("peptoads") were synthesized through multi-step sequences, and their physicochemical properties were studied by X-ray analysis, solubility studies, surface tensiometry, and molecular dynamics simulations. One peptoad (namely peptoad **G**) was investigated for its capacity to solubilize eleven poorly water-soluble drugs by the shake-flask method, and the solubilizing mechanism was explored by molecular dynamic simulations. Additionally, the in-vitro toxicity of peptoads was evaluated on rat macrophage cell lines (RAW 264.7) by MTT assay.
Results: Peptoads possessing short carbon chains (seven carbons) are highly surface-active. Peptoad **G** can solubilize poorly water-soluble drugs very efficiently, by enhancing the water solubility ranging from about 20 to 1100-fold for eleven drugs tested. Molecular dynamic simulations on solubilizing paclitaxel by peptoad **G** showed that peptoad molecules, hydrogen-bonded to the drug, surround the drug with hydrocarbon chains that embed themselves into the interior of peptoad "clumps". It is by this mechanism that paclitaxel is solubilized. Additionally, the in-vitro toxicity of peptoad may be comparable to Cremophor EL and Tween 80.
Conclusions: The colloidal structure of peptoads, along with their solubilizing capacity on poorly water-soluble drugs, their unique solubilizing mechanism and their low toxicity, makes them promising drug-solubilizing agents.

The combined use of HPLC, Gel-LC-MS/MS and microarray in monitoring product quality and safety for gene therapy

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Gene therapy has a unique potential for treating genetic diseases and is now regarded as an indispensable addition in conventional medicines. Quality and safety assessment of gene therapy products presents a great challenge, because they are cell products as well as multi-gene products, including viral and therapeutic proteins and modified cells. In this presentation, we will review our collective experience of using a Gel-LC-MS-MS/MS analysis and the gene-array technology to evaluate quality of HPLC purified products and, to assess the contribution of a range of potential components presented in a final production in product potency and safety in vitro and in vivo systems. The identification of protein biomarkers, transcriptional and toxicogenomic markers using the advance Gel-LC-MS-MS/MS analysis and gene-array supports the development of robust and product-specific assays for monitoring the quality, potency and safety of complex products for gene therapy.

Competition of Sulindac with Specific Markers for the Major Binding Sites on Human Serum Albumin Studied by High Performance Liquid Affinity Chromatography

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Background: The binding of drugs to human serum albumin (HSA) is extensively studied because of the clinical significance of binding and displacement interactions. To predict the binding behavior of one drug in the presence of others and to enable their safe use it is necessary to have knowledge about the nature of drug-protein interactions. Aims: 1) Quantitative characterization of sulindac (SUL) binding to HSA. 2) Study of the competition of SUL with drugs, specific markers for both major binding sites on HSA: phenylbutazone (PBZ) and warfarin (R-WAR and S-WAR) for Site I, and diazepam (DAZ) for Site II. 3) Clarification of the molecular basis of the binding process using thermodynamic approach.
Method: The experimental technique is based on displacement chromatography on HSA-immobilised column. The drug is injected onto the column, and its capacity factor k' is a measure of the binding to HSA. It is influenced by adding a cobinding agent to the mobile phase. A new mathematical approach is used to calculate the affinity constants at several temperatures. The major thermodynamic parameters (free energy change ΔG^0, free enthalpy change ΔH^0, and free entropy change ΔS^0) are calculated.
Results: SUL binds to HSA with high affinity constant ~10^6 M^{-1}. The cobinding of SUL and PBZ does not affect the binding parameters. Both ΔH^0 and ΔS^0 are negative. R- and S-WAR influence differently the binding of SUL. In the presence of R-WAR the affinity constants of SUL are lower. The complexation is accompanied by small negative ΔH^0 and positive ΔS^0 suggesting different kinds of interactions. The cobinding of SUL and S-WAR does not affect the binding behavior. The cobinding of SUL and DAZ results in competition for two types of binding sites: high affinity ~ 10^6 M^{-1} and low affinity ~ 10^4 M^{-1}.
Conclusions: 1) SUL binding to HSA is dominated by hydrogen bonds. The high negative ΔH^0 is the driving force for SUL binding. 2) The cobinding of SUL and PBZ is simply competitive. 3) The binding affinity of SUL is significantly decreased by R-WAR. Conformational changes in HSA may be assumed resulting in the alteration of the binding mechanism. 4) The binding of SUL and S-WAR is simply competitive involving the same kind of interactions. 5) DAZ significantly affects SUL binding developing another type of binding site, dominated by hydrophobic interactions.

Pituitary Resistance To Thyroid Hormone

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Background: Ultrasensitive arrays for determination of TSH remains the milestone for the assessment of thyroid function. However, there are several situations when TSH concentration does not predict the real function of the thyroid. Thyrotropinoma and resistance to thyroid hormone remains the most common causes of the "Inappropriate TSH secretion".
Aim of study: Differential diagnosis between thyrotropinoma and resistance to thyroid hormone.
Results: We present a case of a 24-year-old woman who suffered from mild thyrotoxicosis and diffuse goiter for several years. She had elevated fT3 and fT4 with slightly elevated TSH concentration. Pituitary adenoma was excluded as MRI showed normal pituitary gland, alpha-subunit was normal and TSH concentration raised after TRH administration. Sonography revealed normoechogenic, enlarged thyroid gland. Previously, she was given thiamazole, but without any significant amelioration. Thus, the diagnosis of the syndrome of pituitary resistance to thyroid hormone was established. The patient was given bromocriptine at a dose of 10 mg per day. After 2 months of treatment she achieved a state of euthyreosis.
Conclusions: A single TSH concentration measurement may be unreliable. Bromocriptine is efficient in patients with pituitary resistance to thyroid hormone.

Proline Specific Peptidases as New Targets for Drug Development. Pharmacology and Pathophysiology

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Background: Enzymes hydrolyzing peptide bonds involving proline are of particular interest because of the significance of this imino acids in the determining the conformation of peptide chains. Recent studies have shown that prolyl endopeptidase (PE) and dipeptidylpeptidase IV (DP-IV) participate in the metabolism of proline-containing neuropeptides related to pathogenesis of Alzheimer's disease, Parkinsonian syndrome, muscular dystrophies and other denervating diseases. The aim of our study was to investigate the antidepressive, antiamnestic and antihypoxic properties of PE and DP-IV inhibitors.
Methods: Forced swimming test was used for antidepressive activity studies. The investigation of age related mental decline, motor behavior and estimation of PE levels in different brain regions (cortex, hippocampus, hypothalamus) was carried out in Wistar rats of different age (3 - 24 month old). Hypobaric and acute hypoxic hypoxies were used for study DP-IV inhibitors.
Results: The administration of the PE inhibitors reduces the duration of immobility and potentiates apomorphine-induced stereotyped behaviors. Strong correlation between Ki values and antidepressive and antiamnestic activities of tested inhibitors were revealed. PE inhibitors mimicked the effects of antidepressants. PE level significantly rose in a brain rats 18- and 24 month old. A reduction of learning ability in the active avoidance test and a decline of performance in the reflex of passive avoidance by rats 16-24 month old was shown. Strong correlation between cognitive impairments, neurological deficiency and brain PE level was found. All tested PE inhibitors protected animals from scopolamine-induced amnesia in a rat passive avoidance test. DP-IV inhibitor reduced animal mortality during hypobaric hypoxia, and injected intracisternally changed ventilatory response to acute hypoxia.
Conclusions: 1) The pharmacological activities of PE and DP-IV inhibitors appears to be mediated via possible accumulation of neuropeptides in the brain. 2) The biochemical and pharmacological investigations of PE and DP-IV inhibitors may lead to appearance of a new class of psychotropic drugs with antidepressive, antiamnestic and antihypoxic properties.

Paroxetine-induced CYP2D6 Phenotype Conversion and Clinical Outcomes

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Background: Selective serotonin reuptake inhibitor paroxetine is often prescribed antidepressant in depressive and/or anxiety disorders. Metabolised largely by the polymorphic cytochrome P4502D6, paroxetine exhibits substantial variation in activity among patients. Paroxetine has an inhibitory effect on isoenzyme CYP2D6, this can result in a phenotype conversion to the poor metabolic (PM) phenotype. Paroxetine therapy may be associated with incidences of adverse effects due to an inhibition of its own metabolism. Aims: effect on drug toleration and drug efficacy during paroxetine therapy.
Methods: Subjects: Part A: Influence of paroxetine treatment on phenotype conversion from extensive metabolisers (EM) to PM (*n*=55). Part B: Influence of CYP2D6 phenotype on clinical response in acute paroxetine treatment (*n*=16). Part C: Influence of phenotype on the efficacy and the adverse effects in the maintenance paroxetine treatment (*n*=55). Dextromethorphan test was used for the determination of the metabolic activity of CYP2D6. Genotype determination of patients is based on the detection of polymorphisms in CYP2D6 gene(exons *3,*4,*5,*6). The Hamilton Anxiety Scale (HAMA) and Clinical Global Impression-Severity of Illness Scale (CGI S) were used for clinical response. The Arizona Sexual Experiences Scale (ASEX) was used for sexual dysfunction examination. UKU scale (Utvalg for Kliniske Undersøgelser) was used for examination of adverse effects.
Results: Part A: The effect of paroxetine treatment on CYP2D6 phenotype: in 55 patients sample 34 patients with homozygote EM genotype were found. In 20 of them, PM phenotype was assessed. Part B: In general, earlier onset of the effect of therapy in the acute study (n=16) was observed. Part C: No variation in therapeutic efficacy and adverse effects between PM and EM was found in the maintenance treatment (n=55). One unique output of the study is the variance in the incidence of sexual dysfunction depending on CYP2D6 activity in the females.
Conclusions: 1) PM phenotype patients are more vulnerable to the paroxetine treatment. 2) CYP2D6 activity testing is recommended in all cases with an unexpected therapeutic response. 3) Predicting sexual dysfunction based on the metabolic phenotype and gender during treatment with CYP2D6 inhibitors could be recommended.

Chemoembolisation Using Drug Eluting Beads: Magic Bullets Targeted by Arterial Guidance

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Transarterial chemoembolisation (TACE) is used in the treatment of hypervascularised tumours such as hepatocellular carcinoma (HCC) and involves delivering a drug into the hepatic artery followed by occlusion of the artery with an embolisation agent to starve the tumour of oxygen and nutrients. Although randomised studies have demonstrated a survival benefit for TACE (Llovet *et al.* 2002), the procedure varies vastly in clinical practise leading to inconsistent outcomes. Drug eluting beads (DEB) have been developed from sulfonate-modified polyvinyl alcohol hydrogel microspheres. These devices are capable of targeting tumours with drugs by flow-directed delivery down the feeding arteries and subsequent embolisation of the tumour capillary bed. The DEB affect intra-arterial delivery of chemotherapeutic agents over a sustained period in a controlled manner; deliver a high concentration of drug local to the site of the tumour; reduce the systemic exposure to free drug; occlude the tumour arterial blood supply. DEB have been well characterised *in vitro* with respect to loading and elution of drugs such as doxorubicin and the effects on the physical attributes of the beads related to catheter delivery (Lewis *et al.* 2006a; Gonzalez *et al.* 2007; Lewis *et al.* 2007). These drug-device combinations have been evaluated in a number of pre-clinical models that demonstrate the concept of high local delivery of the drug combined with lower systemic exposure (Hong *et al.* 2006; Lewis *et al.* 2006b). These data are supported by encouraging preliminary phase I/II clinical results in the treatment of HCC (Varela *et al.* 2007). This presentation will provide an overview the bench-to-bedside development of these drug-device combinations for the treatment of liver cancer.

Possibilities of Nonconventional Application of Streptokinase: Studies at the Molecular and Cellular Levels

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Background. Traditionally the preparations of streptokinase (SK) are used in clinical medicine for dissolution of thrombi, fibrin deposits.

In 1987-1991, we described pronounced superoxide dismutase-like activity of SK in chemical systems of superoxide radical generation, the inhibition of plasminogen-activating ability of SK by scavengers of superoxide radical (nitrotetrazolium blue, adrenaline), nucleotides (ATP, cAMP and NAD at rather high concentration: ≥ 0.01 M).

In 1993 we proposed the SK-containing composition for the treatment of patients with long-nonhealing wounds (a patent of the Russian Federation). Its local application in several surgical clinics of Minsk has yielded good results – the healing of such wounds was achieved in all cases.

In 1991, instillation of the SK solutions in eyes was used for treatment of guinea pigs with experimental herpetic keratoconjunctivitis (a patent of the USSR).

Since 1999, on the transplanted cells cultures of rat glioma C6 and human neuroblastoma IMR-32 the mitogenic effect of SK (10^{-11}–10^{-7} M), its stimulation of neuroblastoma cells as well as changes of intracellular contents of DNA, RNA and protein have been demonstrated. The exposition of rat pheochromocytoma PC12 cell culture with 5·(10^{-10}–10^{-6} M) only during 20 min was accompanied by significant changes of the level of ATP- and Ca^{2+}-activated intracellular proteolysis.

On organotypical and dissociated cultures of rat neocortex, sensory (spinal) and vegetative ganglia (on the nutrient mediums with deficiency of blood serum proteins), the neurotrophic effect of SK was established. It manifested itself by maintenance of vital activity of cells at deprivation of blood serum proteins, cold stress, damaging effect of anionic form of ATP, and stimulation of proliferation of glia, Schwann cells and other cells.

The cleavage of bovine fibrinogen in a thin layer of agar gel by washed cells of *Pseudomonas aeruginosa* hospital strain (grown up on plain agar) was inhibited by 20-100 % after SK additions to the cells on.

Conclusions. The scope of the results creates real preconditions for expansion of the medical application sphere of SK preparations, first of all, for stimulation of tissues' regeneration and, perhaps, for treatment of neurological pathology.