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Abstracts Lectures

Acceptance Speech for the Nobel Prize in Medicine and Physiology 1962 Swedish Academy, Stockholm Concert Hall, 10 December 1962: Discovery the Double Helix of DNA

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Your Majesties, Your Royal Highnesses, Your Excellencies, Ladies and Gentlemen.

Francis Crick and *Maurice Wilkins* have asked me to reply for all three of us. But as it is difficult to convey the personal feeling of others, I must speak for myself. This evening is certainly the second most wonderful moment in my life. The first was our discovery of the structure of DNA. At that time we knew that a new world had been opened and that an old world which seemed rather mystical was gone. Our discovery was done using the methods of physics and chemistry to understand biology.

I am a biologist while my friends *Maurice* and *Francis* are physicists. I am very much the junior one and my contribution to this work could have only happened with the help of *Maurice* and *Francis*. At that time some biologists were not very sympathetic with us because we wanted to solve a biological truth by physical means. But fortunately some physicists thought that through using the techniques of physics and chemistry a real contribution to biology could be made. The wisdom of these men in encouraging us was tremendously important in our success. *Professor Bragg*, our director at the Cavendish and *Professor Niels Bohr* often expressed their belief that physics would be a help in biology. The fact that these great men believed in this approach made it much easier for us to go forward.

The last thing I would like to say is that good science as a way of life is sometimes difficult. It often is hard to have confidence that you really know where the future lies. We must thus believe strongly in our ideas, often to point where they may seem tiresome and bothersome and even arrogant to our colleagues. I knew many people, at least when I was young, who thought I was quite unbearable. Some also thought *Maurice* was very strange, and others, including myself, thought that *Francis* was at times difficult. Fortunately we were working among wise and tolerant people who understood the spirit of scientific discovery and the conditions necessary for its generation.

I feel that it is very important, especially for us so singularly honored, to remember that science does not stand by itself, but is the creation of very human people. We must continue to work in the humane spirit in which we were fortunate to grow up. If so, we shall help insure that our science continues and that our civilization will prevail. Thank you very much for this very deep honor.

Discovering the Double Helix

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Key to the finding of the double helix was the realization on the morning of February 28, 1953 that A-T and G-C base pairs have appropriate shapes for holding together the two chains of the DNA molecules as a regular double helix. The two glycosidic bonds of a purine-pyrimidine pair are not only the same distance apart in the A-T and G-C pairs but are related to each other by a diad and

can thus be fitted in the double helix either way around. This feature allows ail four bases to occur in both chains. Base pair symmetry, moreover, dictates that the two chains run in opposite 5'-3' and 3'-5' directions.

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In vitro and *in vivo* Evaluation of a Stable GnRH Analogue for the Treatment of Prostate Cancer and Endocrine Disorders

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Gonadotropin-releasing hormone (GnRH - l) receptor agonists are widely used in the clinic for the treatment of prostate cancer and endocrine disorders. However, such agonists are characterized by poor pharmacokinetic properties due to their peptidic nature, often requiring repeated administration or special formulations. Therefore, the development of novel peptide analogues with enhanced *in vivo* stability could potentially provide therapeutic alternatives. The pharmacological evaluation of a bioactive peptide [Des-Gly^{10}, Tyr^{5}(OMe), D-Leu^{6}, Aze-NHEt^{9}]GnRH, analogue 1, is presented herein and compared with leuprolide. Peptide stability was evaluated using mouse kidney membrane preparations, followed by a liquid chromatography-tandem mass spectrometry-based approach that afforded identification and quantification of its major metabolites. The analogue was significantly more stable *in vitro* in comparison with leuprolide. *In vitro* and *in vivo* stability results correlated well, encouraging us to develop a clinically relevant pharmacokinetic mouse model, which facilitated efficacy measurements using testosterone as a biomarker (pharmacologic response). Analogue 1, an agonist of the GnRH receptor with a binding affinity in the nanomolar range, caused testosterone release in mice that was acutely dose-dependent, an effect blocked by cetrorelix (GnRH receptor antagonist). Repeated dosing studies in mice demonstrated that analogue 1 was well tolerated and had potency similar to that of leuprolide, based on plasma and testis testosterone reduction and histopathological findings. Analogue 1 also shared with leuprolide similar significant antiproliferative activity on androgen-dependent prostate cancer (LNCaP) cells. On the basis of pharmacokinetic advantages, we expect that analogue 1 (or analogues based on this new design) will be therapeutically advantageous for the treatment of cancer and endocrine disorders.

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Impact of Nanoscience on Biomedical Applications: Fighting Cancer

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Advances in manipulation and understanding the properties of nanomaterials have resulted in new and promising methods in biomarker detec-tion, tissue imaging and therapy. This has pro-pelled research towards a multidisciplinary ap-proach of disease treatment and of cancer in particular, since cancer remains amongst the most frequent causes of death in the developed world. The effectiveness of conventional treat-ment and drug administration technologies has often been limited by drug resistance of cancer cells and severe side effects on normal cells. Therefore, one of the major challenges is the targeted and controlled delivery of therapeutics in order to suppress their systemic distribution and the restriction of drug-resistance development.

Nowadays, advances in nanoscience and nanotechnology provide new tools to tackle such issues and increase the treatments' efficacy through the utilization of nanomaterials (metals, semiconductors, magnetic phases or carbon), which have been specially engineered into smart/multifunctional hybrid drug nanocarriers. Representative examples and results from this research field will be discussed in the presentation.

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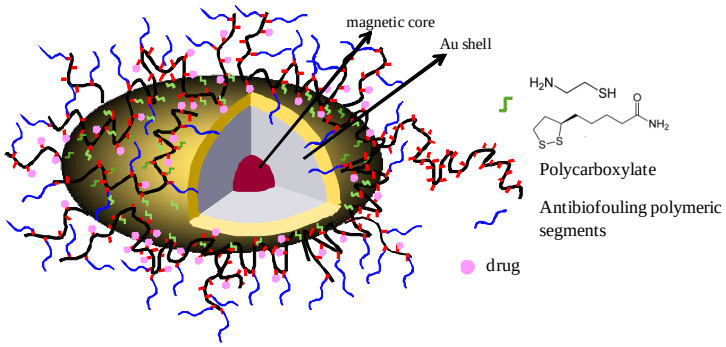

The Protease-activated Receptor 1 Possesses a Functional and Cleaved Signal Peptide which is Essential for Receptor Biosynthesis and mRNA Expression

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The Protease-activated receptors (PARs) are a subfamily of G-protein coupled receptors that are activated by cleavage of their extracellular domain by serine proteases such as thrombin and trypsin. These proteases cleave the amino terminus of the receptor, which in turn uncovers a tethered ligand that transactivates the receptors. PARs are highly expressed in platelets, but also in endothelial cells, myocytes and neurons. There are four (4) known PARs (PAR_1, PAR_2, PAR_3 and PAR_4). In the case of the PAR1 receptor, it is known that thrombin cleaves between amino acid 41-42 in its extracellular domain to activate the receptor. According to prediction programs, the PAR1 receptor also possesses a cleavable signal peptide in its amino terminus (residues 1-21) which might mediate targeting of the receptor to the membrane of the endoplasmic reticulum (ER); the first step of the intracellular transport of the receptor. However, it was unknown whether this signal peptide is functional.

We show for the first time that the PAR1 receptor indeed contains a functional signal peptide which mediates ER targeting and insertion of the receptor and is cleaved following the ER insertion process. Moreover, the signal peptide is essential for establishing a functional receptor at the plasma membrane (receptor biosynthesis). Furthermore, it seems that the signal peptide stabilizes the mRNA secondary structure and consequently the transcription levels of the receptor since its absence leads to non or low expression rates.

Regulatory T Cells (Tregs) in Cancer Patients: Preliminary Data

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Tregs play a pivotal role in immune homeosta-sis by suppressing the proliferation and function of effector T (Teff) lymphocytes, as well as other immunocytes. Thus, they are implicated in various clinical entities, where the regulation of immune responses might be relevant, including allergies, infections, autoimmune diseases, and tumor immunity. In cancer development tumor-associated antigens are presented to the immune system by professional antigen-presenting-cells priming naïve-T-cells to become antigen-specific $CD8^+$-cytotoxic-lymphocytes that, though, are unable to reject the tumor (1,2). These cells are far less effective than expected possibly due to an immunosuppressive effect counteracting the anti-tumor immune responses addressed by Tregs (3). Though, the key elements in the interaction of host anti-tumor immunity, tumor immunopathology and therapy are still not well understood. To examine the functional capacity of Tregs in the periphery of patients with cancer, before and after receiving chemotherapy (CTX), peripheral blood samples were collected from cancer patients receiving per-os (PO) CTX, Teff and Treg populations were isolated and their suppressive and proliferative activity were measured. Our preliminary data indicate that PO-CTX in cancer patients results in a decline in the Treg/Teff ratio, while Treg suppressive capacity tends to decrease and Teff proliferative activity to increase, after such therapeutic schemes. Further investigation of a possible role of CTX schemes in anti-tumor immune responses is required.

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Exploring Peptide/Protein Interactions in Immunogical Complexes with Molecular Dynamics Simulations

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Discovering new effective drugs is one of the biggest scientific challenges of our time. This target has become a field for joint efforts from both industry and academia as well as from both public and private sectors of the society. Molecular dynamics simulation is a valuable tool towards an understanding of the complex structure of biological systems, especially in the study of the flexibility of the biological molecules such as peptides or proteins (1). A short but in-depth review of recent applications of MD simulations in immunology will be discused in the current presentation. For example, the dynamics of the pMHC/TCR interactions (2), some new insights on peptide/H-$2K^b$ interactions (3) or how TCR binding of a peptide/MHC complex can alter the cis/trans equilibrium of a peptide bond at the pMHC/TCR interface (4). Moreover, some recent advances of the antibody/protein interactions will be highlighted (5).

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The Role of Microglia in Central Nervous System Inflammation

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Microglia has been traditionally considered as a destructive cellular component of inflammatory and degenerative processes throughout the Central Nervous System (CNS), leading to demyelination and possibly axonopathy in both Multiple Sclerosis (MS) and the animal model of the disease, the Experimental Autoimmune Encephalomyelitis (EAE). However, recent studies indicate that transition of resting to activated ramified microglia is not per se neurotoxic process but may be neuroprotective-one, as well. Since microglia is a highly dynamic cell, reactive to almost any tissue changes, it can support repair and tissue reconstruction in the presence of anti-inflammatory cytokines (IL-4, IL-13, IL-10 and TGF-β) (Colton et al 2009, Napoli and Neumann 2010). In addition, peripheral macrophages when activated, may acquire either the *classical, proinflammatory* activation state (inducing the iNOS pathway - M1 type) or the *alternative, anti-inflammatory* state (inducing the Arginase-1 pathway - M2 type), the latter being reparative/anti-inflammatory. Most importantly, a recent study indicates that the M2 ratio determines the EAE severity, i.e. high M2-ratio thereby resulting in mild EAE and vise versa (Mikita et al 2011). In addition, experimental data from our laboratory indicate for the first time that via appropriate interventions increased percentage of M2 macrophages/microglia in EAE is feasible (Lourbopoulos et al, unpublished observations). Therefore, our data, along with others, support a new possible therapeutic/immunomodulating target for EAE and MS, beyond the one of Th1/Th2 lymphocytic shift, ie the regulation of M1/M2 macrophages.

Study of the Immunomodulatory Effects of Myelin Peptide Analogs in Multiple Sclerosis (MS)

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MS is a progressive degenerative disease of the central nervous system (CNS), characterized by local macrophage and T-cell infiltration and autoantibody production that lead to demyelination and loss of neurologic function. MS is an autoimmune disease triggered by Th1 and Th17 $CD4^+$ T helper cells that secrete pro-inflammatory cytokines, mainly IFN-γ, TNF-α and IL-17. Moreover, MS is characterized by a reduction in the populations of $CD4^+CD25^+Foxp3^+$ and Th2 (IL-4) /Th3 (TGF-β) /Tr1 (IL-10) regulatory or immunosuppressive cells. Thus, in MS there is an imbalance between the effector and suppressor arms of the immune response, and an effective therapy would result in correcting this imbalance, shifting the pro-inflammatory Th1/Th17 cell/cytokine profile to the anti-inflammatory Th2/Th3/Tr1. Current therapeutic approaches in MS include the design and use of peptide epitopes of myelin such as myelin basic protein (MBP), proteolipid protein (PLP) and myelin glycoprotein of oligodendrocytes (MOG), as immunoregulatory drugs.

In this study, we tested *in vitro* the activity of peptide analogs of myelin conjugated or not with mannan. These peptides have been studied *in vivo* in a rat animal model of experimental autoimmune encephalomyelitis (EAE) to evaluate their toxocity and effect on disease progression, with positive results. More specifically, we tested the effect of 1 MBP and 2 MOG peptide analogs on the quantity and function of various T regulatory cell populations in cultures of peripheral blood mononuclear cells (PBMC) from MS patients and healthy subjects (controls), and on the secretion patterns of type-1, pro-inflammatory cytokines (IL-2, IL-17A, TNF-α, IFN-γ, IL-6) and type-2, anti-inflammatory cytokines (IL-4, IL-10, TGF-β). Results from 28 patients and 18 controls showed that 2/5 peptides (at a concentration of 10 pg/ml/10^6 PBMC) had a positive effect on PBMC from patients receiving natalizumab treatment (N=14). Culture with these peptides, resulted in a decrease of the Th1 and Th17 responses, a reduction of ratio of type-1/type-2

cytokines, and an increase in Treg numbers and function. In contrast, the same peptides augmented the Th1 and Th17 responses of control PBMC and, also, increased the type-1/type-2 cytokine ratio of control PBMC.

Deciphering Genetic Disease in the Genomic Era: The Model of GnRH Deficiency

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The neuroendocrine mechanisms regulating the onset of puberty and conferring reproductive capacity remain one of the major unsolved biological mysteries. The onset of sexual maturation and the acquisition of reproductive capacity are associated with the activation of the hypothalamic-pituitary-gonadal (HPG) hormonal axis during puberty. Gonadotropin-releasing hormone (GnRH) serves as the *pilot light of reproduction*: its pulsatile secretion from hypothalamic neurons stimulates the pituitary gonadotropes to release gonadotropins. These in turn promote sex steroid production and gametogenesis in the gonads, bringing about the phenotypic changes associated with sexual maturation as well as the ability to reproduce. Patients with congenital isolated deficiency of GnRH afford translational researchers a unique opportunity to uncover genes and signaling pathways responsible for sexual maturation and reproduction. Although rare (1/10.000 people) GnRH deficiency has proven to be a prismatic disorder that has facilitated the discovery of more than a dozen genes implicated in the neuroendocrine control of reproduction. Traditionally, GnRH deficiency was considered a simple monogenic disorder caused in each patient by a single genetic defect affecting a specific process in the activity of the HPG axis. These simplistic notions were surpassed in the last few years through our investigations showing that: (i) Patients with GnRH deficiency can each have defects at multiple levels of the HPG axis. (ii) GnRH deficiency is a complex genetic disease with each patient harboring multiple genetic defects across different disease-associated genes. (iii) Genetic defects linked to GnRH deficiency are also present in women with functional hypothalamic amenorrhea, a common, milder and acquired form of GnRH deficiency. These advances have implications for elucidating the neuroendocrine control of reproduction but also for modeling the genetics of other complex diseases.

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Crucial Role of Granulocytic Myeloid-derived Suppressor Cells in the Regulation of the Central Nervous System Autoimmune Disease

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One of the main unresolved issues in autoimmune diseases is the restoration of immune homeostasis and self-tolerance. Although the variety of therapeutic targets is enormous, a large number of patients with autoimmune syndromes fail to either respond to current therapy or to achieve long-lasting remission after its cessation. The goal therefore, would be to develop novel therapeutic protocols that could cure and not only palliate autoimmunity, by resolving inflammation and establishing lasting tolerance. Myeloid-derived suppressor cells (MDSCs) encompass a newly described population of cells that potently suppress immune responses, rendering them an attractive target for cell-based therapy in autoimmune diseases. Extensive studies have explored the role of MDSCs in the regulation of immune responses during cancer and infections. However, their potential in regulating autoimmune responses, and most importantly their contribution in human autoimmunity remain elusive. Herein, we demonstrate that MDSCs are enriched in the periphery of subjects with active MS, and largely accumulated prior to disease remission, in the peripheral lymphoid compartments of mice with experimental autoimmune encephalomyelitis (EAE). Importantly, MDSCs from MS patients suppressed the activation and proliferation of autologous $CD4^+$ T cells *ex vivo*. Similarly, the granulocytic subset of mouse MDSCs suppressed encephalitogenic Th1 and Th17 immune responses, significantly delayed EAE onset and ameliorated EAE upon *in vivo* transfer. Both human and mouse MDSCs upregulated expression of the inhibitory molecule programmed death 1 ligand (PD-L1), with the latter exerting their suppressive function in a PD-L1-dependent fashion. Collectively, these data reveal a pivotal role of MDSCs in the natural regulation of multiple sclerosis and has implications in the treatment of autoimmune disease.

Drugging Undruggable Targets: Sculpting Ligand Selectivity for Difficult Oncogenic Targets

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We currently witness a decline in the development of efficient new anticancer drugs, despite salient efforts and rigorous research conducted by academic institutions and pharmaceutical industry worldwide in all fronts of cancer drug discovery. Given the inherent complexity of cancer biology, it is now considered that modern cancer drug discovery should optionally be shifted from traditional targets (enzyme or receptors) to uncharted areas of so called *undruggable targets*. To challenge this we have established a three dimensional approach: We start with an *in silico* systems-biology protocol to define key features discriminating cancer-related proteins from non-cancer proteins. We then use an *in silico* approach to identify lead molecules from in house or commercially available small molecule libraries. We then screen the best candidates through a battery of biochemical and biophysical assays. Optimization of the lead molecules follows through modern chemoenzymatic approaches. Successful examples will be presented from our recent work.

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Optical Detection of Specific DNA and RNA Sequences *via* Nanoparticle-Based Dipstick Tests

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Currently there is a growing interest in the development of dipstick tests for nucleic acid analysis (DNA and RNA) that enable visual detection of the target sequence. These tests are simple, do not require special equipment or highly quailfied personnel, while eliminate the several necessary washing and incubation steps. The molecular recognition through hybridization represents the most appropriate technique for nucleic acid detection. The visual detection through the naked eye, with the dipstick test is based on strongly colored particles, such as gold, polystyrene or carbon nanoparticles and is completed within a few minutes, 10-15 min. We have developed dipstick tests using all the three types of particles for the detection and confirmation of target DNA or RNA, *via* hybridization. More specifically, gold nanoparticles, colored (red or blue) polystyrene particles and carbon nano-strings are functionalized with oligonucleotide probes that are specific for the target analyzed or a specific antibody and used as reporters for the optical detection of the target. Flowing through a diagnostic membrane, these reporters are captured on a capture line, in the presence of the target and a visual result is achieved, by the formation of a colored line, within a few minutes. A second colored line is formed to confirm the proper function of the sensor. The dipstick test is applied for the optical detection of single-stranded DNA, double-stranded DNA, RNA and visual genotyping of Single Nucleotide Polymorphisms (SNPs) in human genomic DNA.

The Role of Modern Experimental Biomedical Research in the Development of New Products and Therapies with Clinical Applications

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Drug development in the medical arena is dependent on a variety of support structures. The introduction of a new molecule or drug ideally begins with innovation responding to clinical need. Along the way, the drug is subject to scientific, clinical, engineering, and regulatory scrutiny and may be lost to the target community by recognition of a lack of validity, safety, or effectiveness, or by mismanagement of its development. It is critical to understand the method by which new drugs or therapies with clinical applications are funded in the period between idea and product launch.

1. *Seed stage*: A logical mechanical, physiologic and engineering approach to well-defined clinical problem and need for which current solutions remain imperfect.
2. *Early stage*: proof of concept as represented by computer modeling, prototype development with in vitro testing or early animal validation.
3. *Pre-clinical stage*: demonstrated safety and efficacy in animal models with progression to phase I testing demonstrating safety in humans.
4. *Pre-launch stage*: phase 2 and phase 3 trials demonstrating efficacy and ultimately effectiveness under tightly controlled circumstances.

Research is a daily lesson of strategy, of personal and team responsibility. Lesson of Life!

Personalized Cancer Treatment in the Genomic Era

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Major advances in basic science have created an opportunity for significant progress in the treatment of cancer. Several genomic approaches have affected multiple aspects of cancer: tumor classification, prognostic markers, predictive indicators of drug response, the development of new

drug therapies, strategies for monitoring disease, and susceptibility to cancer.

The advent of microarray-based profiling has substantially increased the power to subclassify cancers. In breast cancer this has contributed to the development of a classification with the different subtypes having markedly different clinical and biologic features, including patient survival.

The identification of oncogenes involved in the initiation and progression of tumors has generated targets for the development of new anticancer drugs. Several new agents, small molecules, and monoclonal antibodies directly affecting oncogene products have been developed. Considerable progress has been made in producing small molecules capable of inhibiting the enzymatic activity of ABL, KIT, EGFR, and ERBB2. New effective treatments are available for many solid tumors, including breast cancer, non-small cell lung cancer and gastrointestinal stromal tumors. As the therapeutic agent is targeted at a particular cellular protein, genomic alterations in the gene encoding that protein can be major determinants of response.

All the above have led to the implementation of targeted treatments according to the molecular profile of the tumor, and in some occasions, according to the patient's genetic characteristics, leading to the development of personalized cancer medicine. The success of personalized medicine depends on having accurate diagnostic tests that will identify patients who may have benefits from targeted therapies.

Multiple Sclerosis Therapy: Present and Future Agents

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Multiple Sclerosis (MS) is an inflammatory, autoimmune disease of the central nervous system, in which autoreactive T-cells cross the blood-brain barrier and trigger an immune cataract that leads to demyelination, axonal damage and accumulation of neurological deficit. Treatment of MS consists of treating acute exacerbations with high doses of methylprednizolone for a few days, symptomatic relief of complications (e.g. spasticity, bladder dysfunction, paroxysmal symptoms, etc.) and prophylactic treatment with disease modifying therapies (DMTs) that aim at immune regulation and attenuation of future relapses. Currently available prophylactic therapies include the interferons (IFNb-1a and IFNb-1b), glatiramer acetate, the monoclonal antibody natalizumab and mitoxandrone. After almost 2 decades of use of these agents, all in parenteral form, we have the advent of oral therapies, with two new drugs, fingolimod and cladribine. Fingolimod has been approved by the FDA on September 2010 and in Europe on January 2011 for the treatment of relapsing- remitting MS. In the meantime, many other treatments for MS are being tested with encouraging results, providing new perspectives for MS therapy in the future.

Transdermal Drug Delivery: New Sartan Perspective

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Hypertension is an important disease since a high percentage of the population presents high levels of blood pressure. It is an independent risk factor for congestive heart failure, myocardial infaraction, renal failure and strokes.

In the market are several transdermal therapetic systems (TTS), known as patches for the treatment of Alzheimer (Rivastigmine-Exelon), pain (Fentanyl-Durogesic), parkinson disease (Rotigotine-Neupro), hormone regulation (Estradiol-Estraderm) etc.

To date there are various drugs for the treatment of hypertension but none of them is administered transdermally (beta-adrenergic receptor blockers in combination with diuretics, ACE inhibitors or other drug therapy).

Transdermal delivery presents many advantages compared to other routes of administration. Administration with transdermal delivery presents stable release through skin as well as stable lev-

els of drug in plasma. Furthermore, the first pass effect is avoided and long duration of drug release could be achieved, depending on the formulation of the patch. Consequently, transdermal delivery is a novel route of administration in Modern Medicine and a safe antihypertensive treatment with long duration.

During the last decade, in our laboratory we have developed several methods for the design, synthesis and biological evaluation of specific and selective non peptide AII receptor antagonists for the treatment of hypertension with transdermal delivery. These analogues are selective AT1 receptor antagonists of Angiotensin II (AII) and *in vivo* protocols shows efficient transdermal penetration using different formulations.

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Peptide Mimetics a New Generation of Drugs: Fighting Multiple Sclerosis and Hypertension

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The discovery of Losartan a non peptide Angiotensin II Receptor antagonist was announced in 1989 during the Gordon Research Conference on Angiotensin and Renin-Angiotensin-System (RAS). Breakthroughs, in this evolution, was the discovery of Captopril by Miguel Ondetti (1975) and Losartan by Timmermans (1989). In this lecture the main steps followed in our laboratories are mentioned which led to our Sartan, named Elsartan. Briefly the main steps are: (i) Peptide (The tool), (ii) Peptide Model (The ligand – recaptor interaction), (iii) Cyclic Peptide (The drug lead), (iv) Non-peptide mimetic (The Drug). Also the biological steps required for Clinical Trial approval, will be reported.Immunodominant Epitopes of human proteins MBP, PLP, MOG of myelin sheath are implicated in Multiple Sclerosis. Specific Analogues have been found to immune rats rendering them potential therapeutics vaccine drugs in the Immunotherapy of Multiple Sclerosis. Furthermore, our cyclic MBP 83-99 peptides, for the first time to be reported as HLA and MHC binders and more stable compared to linear counterparts, possess a series of important immunomodulatory properties rendering them as putative drugs for treating multiple sclerosis and potentially other Th1-mediated autoimmune diseases. In the light of the results and findings in our research, the main immunodominant peptides and their head to tail cyclic counterparts conjugated to reduced mannan have been selected to constitute a mixture cocktail drug for preclinical investigation in preparation of New Drug Application (NDA) for Clinical Phase I and II studies in the Immunotherapy of Multiple Sclerosis.

Therapeutic Aspects for MS: Are we heading in the Right Direction?

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Several disease-modifying therapies are approved for the management of multiple sclerosis (RRMS) that primarily target activated autoreactive T cells and their contribution to MS pathology. By preventing inflammation, demyelination and subsequent axonal damage, these treatments reduce the frequency or relapses to differing extents. Despite the availability of effective

disease-modifying therapies, there is a need for new potential analogues for the immunotherapy of MS. Thus, the design of peptide mutants of disease-associated myelin epitopes to alter immune responses offers a promising avenue for the treatment of MS. Therefore, we designed and synthesised a number of peptide analogues by mutating the principal TCR contacts residue and these peptides were conjugated to reduced mannan. Cellular and humoral responses were measured to identify the best candidate peptide, and molecular modelling was used to reveal new H-bonding and van der Waals interactions between peptide and MHC ($I\text{-}A^{s}$). Current treaments and immunotherapeutic approaches for MS will be discussed and compared to our novel immunotherapeutic strategy.

Mutations in the Erythroid Transcription Factor KLF1 Cause Hereditary Persistence of Fetal Hemoglobin

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Hereditary Persistence of Fetal Hemoglobin (HPFH) is characterized by persistence of fetal hemoglobin (HbF, $\alpha_2\gamma_2$) of over 2% in adults. Ten of 29 members from a unique Maltese family presented with HPFH (HbF: 3%-20%). Genome-wide SNP array and linkage analysis data were combined with expression profile data of HPFH and normal primary erythroid progenitors (HEPs). A candidate region was mapped on chromosome 19p13.12-13 and a nonsense mutation was identified in the *KLF1* gene (p.K288X) ablating the protein's DNA binding domain. Only HPFH family members were p.K288X carriers; p.K288X was absent in the general Maltese population. Expression profiling of HPFH HEPs revealed downregulation of KLF1 target genes, including *BCL11A*. Lentiviral-mediated knockdown of KLF1 and overexpression of full-length and truncated KLF1 in normal and/or HPFH HEPs, as well as ChIP experiments, showed that, in addition to its established role in adult globin expression via the activation of *HBB*, KLF1 is a critical activator of the *BCL11A* gene, which represses fetal *HBG1*/*HBG2* expression in adults. These findings support the notion that KLF1 is a regulator of human fetal-to-adult globin switching. The reactivation of *HBG1*/*HBG2* genes in adults, via attenuation of KLF1 activity, provides novel lead for molecular therapeutics of β-type hemoglobinnopathies. These data, along with similar recent findings on the *KLF1* gene, have been now deposited into HbVar database (http: //lovd.bx.psu.edu/home.php?select_db=KLF1), according to the microattribution approach, which is implemented for the first time in an effort to systematically document all genetic variation data in hemoglobinopathies.

A New Mechanism in Insulin Chain Combination: Fighting Conformational Diseases

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Protein misfolding, mainly of mutated proteins, is the reason for more than 60% of human diseases. Prominent examples are besides aging, diabitis, several cancer types and Alzheimer. Because of the high similarity of the misfolded proteins to the natural proteins these cannot be recognized by the human immune system e.g. from the endoplasmic reticulum associated degradation (ERAD) system. The better recognition of the mutant proteins by the immune system could be achieved by their selective modification, best by their combination with an other peptide or protein. This has not been studied until now probably because of the common opinion that intermolecular protein-protein combination is a rather slow process in the low concentration of the proteins in the cell. In our recent studies for the production of the insulin like peptide relaxin we observed the fastest and more selective itermolecular *in vitro* peptide folding reported to

date. This fast intermolecular folding was observed not only in the case of the two chains of the same insulin like peptides but also between the chains of very different peptides like relaxin and IGF. These observations lead to the postulation of a new mechanism of insulin chain folding. In addition, it becomes obvious, that peptides can be developed which will combine fast with misfolded and mutant proteins scavenging them selectively from the cell. This will lead to the development of a new generation of pharmaceuticals, which will prevent and not cure the diseases.

The Renin-Angiotensin System and the Cardiorenal Syndrome

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The life expectancy for millions of years was about 35 years of age and the people were obliged to work hard, walk for great distance and fight to keep or conquer new lands. They also suffered from diarrhea, as it is the case in our days with people in the Third World. Therefore, during evolution, many systems have been developed capable to maintain the perfusion pressure, retain salt and water by the kidney and restore intravascular volume. Renin-Angiotensin System (RAS) comprises the most important hormonal system for regulating cardiovascular homeostasis. Angiotensin II is the active octapeptide, which causes vasoconstriction, salt retention directly and indirectly via aldosterone, acts as growth factor in a variety of cells, promotes inflammation and coagulation and augments ery-thropoiesis.

After industrial revolution and over the past two centuries the standard of living improved, and a tremendous progress has been seen in the field of science, technology and medicine. The life expectancy increased gradually to over 80 years of age and now the same systems that supported blood pressure in conditions of hypovolemia are responsible for the development of hypertension and are associated with cardiovascular morbidity and mortality. Therefore, the inhibition or the blockage of such systems are used to control hypertension, improve quality of life and improve survival, in patients with myocardial infarction and congestive heart failure, renal disease and diabetes mellitus.

Cardiorenal syndrome is a term used over the past 10 years to describe patients with simultaneous heart and renal disease due to common causes. The basis for the development of this syndrome is the aging of the population and the epidemics of obesity and diabetes. Inhibition of RAS is the basis of the treatment of patients with cardiorenal syndrome. Although the cardiac survival improves, side effects from the use of RAS inhibitors include deterioration of renal function and development of anemia. Studies are under way to evaluate how to adjust doses, maintain renal function and correct anemia in an effort to further improve outcome.

Neurosteroidal Agonists of Nerve Growth Factor (NGF) Receptors with Neuroprotective and Neurogenic Properties

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Neurosteroids, produced by neurons and glia, have been shown to exert strong neuroprotective and neurogenic properties, while their decline during ageing is associated to neurodegenerative diseases. We have shown that endogenous neurosteroid dehydroepiandrosterone (DHEA), protects neuronal cells against apoptosis at nanomolar concentrations (Charalampopoulos et al, PNAS 2004), *via* binding to specific plasma membrane receptors (Charalampopoulos et al, FASEB J 2006), rapid activation of prosurvival kinases MEK1/2/ERK1/2, and PI3K/Akt, the induction of transcription factors CREB and NFkB and the transcriptional activation of anti-apoptotic Bcl-2 genes. Recently, we have described the nature of DHEA membrane binding sites, which in fact are

the receptors of the main neurotrophin, nerve growth factor (NGF) (Lazaridis et al, PLoS Biology 2011). Indeed. DHEA exerts its neurotrophic effects by directly interacting with TrkA and p75NTR receptors of NGF, efficiently inducing TrkA phosphorylation, and NGF receptormediated signaling; Shc, Akt, and ERK1/2 kinases down-stream to TrkA receptors and TRAF6, RIP2 and RhoGDI interactors of p75NTR receptors, preventing the apoptotic loss of NGF receptor positive sensory and sympathetic neurons in *ngf*$^{-/-}$ null mice (Lazaridis et al, PLoS Biology 2011). These findings may have important pharmacological applications in the treatment of neurodegenerative diseases. DHEA cannot be given long term, due to its *in vivo* conversion to estrogens and androgens. On the other hand, polypeptidic NGF does not cross the brain blood barrier (BBB). We have recently synthesized 17 spiro analogs of DHEA with strong neuroprotective effects and deprived of estrogenic or androgenic actions (Calogeropoulou et al, J Med Chem 2009). These synthetic neurosteroids cross the BBB, interact with NGF receptors and mimic various actions of NGF. They are now tested as potential therapeutic agents in various animal models of neurodegenerative diseases.

Achievements and Prospects of Human Genomics in Molecular Medicine

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Μετά την ανακάλυψη της δομής της διπλής έλικας του DNA, υπήρξαν δύο ακόμη κομβικά σημεία στην ιστορία της Μοριακής Βιολογίας, η αξιοποίηση της τεχνολογίας του ανασυνδυασμένου DNA για τον προσδιορισμό της δομής, της οργάνωσης και του ρόλου των γονιδίων σε φυσιολογικές και απλές παθολογικές καταστάσεις, και πιο πρόσφατα η αποκάλυψη της δομής και αρχιτεκτονικής των γονιδιωμάτων με τεχνολογίες υψηλής απόδοσης. Η συσχέτιση χιλιάδων αλληλομόρφων γονιδίων με κληρονομικά και πολυπαραγοντικά νοσήματα έχει αυξήσει εκθετικά τον αριθμό των διαθέσιμων διαγνωστικών μοριακών αναλύσεων και έχει συνεισφέρει στη σταδιακή αποκάλυψη της λειτουργίας των αντίστοιχων πρωτεϊνών, γεγονός που διαμορφώνει τις προϋποθέσεις για σχεδιασμό ολοένα και πιο αποτελεσματικών φαρμάκων. Συγκεκριμένοι τύποι νουκλεοτιδικού πολυμορφισμού και αποκλείσεις στην οργάνωση του γονιδιώματος συσχετίζονται με συχνά εμφανιζόμενες παθολογικές καταστάσεις ή με τη διαφορετική ανταπόκριση των ατόμων σε φάρμακα, οδηγώντας έτσι σε ακριβέστερη διάγνωση εξατομικευμένα σχεδιασμένη θεραπευτική αγωγή και, κατά συνέπεια, βελτιωμένη ιατρική φροντίδα. Η ολοένα πιο λεπτομερής περιγραφή του δικτύου των μοριακών μηχανισμών που διέπουν τον κυτταρικό πολλαπλασιασμό, την ανάπτυξη και τη Φυσιολογία, προσφέρει ιδέες και εισηγείται εφαρμογές νέων καινοτόμων τεχνολογιών. Αυτά τα επιτεύγματα είναι, όμως, μόνον η αρχή. Η μείωση του κόστους αλληλούχησης του DNA, η βελτίωση των *ομικών* τεχνολογιών ανάλυσης της γενετικής πληροφορίας στα διάφορα επίπεδα οργάνωσης και πολυπλοκότητας των βιολογικών συστημάτων με τη βοήθεια της πληροφορικής και της υπολογιστικής βιολογίας, σε συνδυασμό με τα επιτεύγματα της *στηριζόμενης σε υπόθεση* έρευνας, θα επιτρέψει ακόμη πιο ακριβή συσχέτιση του γενετικού και επιγενετικού προτύπου με την κλινική εικόνα και την αποκάλυψη των μοριακών μηχανισμών που διέπουν τις συχνά εμφανιζόμενες ασθένειες, στοιχεία που είναι απαραίτητα για πιο αποτελεσματική θεραπεία.

Honorary Lecture to the Nobel Winner and Emeritus Professor James Watson

Thomas Mavromoustakos

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One of the great discoveries that led to the human knowledge explosion and horizons in the scientific research related to treatment of major human diseases and nanotechnology is without doubt that of the structure of the double helix of DNA (1,2). In this discovery, the honored person

contributed significantly through his hard research work and effort. For this effort, he has been honored with the highest scientific achievement sharing in 1962 the Nobel Prize with F. Crick and M. Wilkins. In this lecture, an emphasis will be given to the factors that led to the DNA structure discovery and the future prospects of the scientific knowledge as a result of this discovery.

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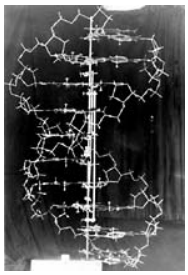

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Differential Regulation CRF_1-mediated Signalling after the Interaction of two Extracellular Residues of Receptor with Different Ligands

K. Gkountelias[1], T. Tselios[2], A. Gravanis[1] and G. Liapakis[1]

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The corticotropin releasing factor (CRF) is a 41 amino acid peptide that plays an important role in modulating the response of central nervous system (CNS) to stress. Dysregulation of CRF neuronal circuits from stressful stimuli is closely associated with the appearance of anxiety and depression. The CNS actions of CRF are mediated mainly through its interaction with the type 1 receptor (CRF_1) and the subsequent activation of different signalling pathways. The CRF_1 consists of seven plasma membrane-spanning segments, connected with three extracellular and three intracellular loops. In this study we found that two residues in the second extracellular loop (EL2-residues) of CRF_1 interact with the amino-terminal residues 9-11 of CRF and the corresponding ones of its related peptide, sauvagine. We also found that in contrast to CRF, the interaction of EL2-residues with the amino acids 9-11 of sauvagine is important for CRF_1-mediated stimulation of cAMP accumulation. These results lead to the hypothesis that the interaction of EL2-residues of CRF_1 with the amino-terminal residues 9-11 of CRF is possibly important for the stimulation of a different than the cAMP signalling pathway. We are now performing experiments to determine the signalling pathways, which are regulated by the interaction of EL2-residues with different peptides of CRF family. These studies will put the basis for the development of CRF_1-selective selective signalling-specific drugs, which could be used for the elucidation of the role of CRF_1 in the pathophysiology of anxiety and depression, and possibly for the treatment of these diseases.

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Synthetic Strategy and Biological Evaluation of Potent Nonpeptide ANG II AT1 Receptor Antagonists Based on 4(5)-Butylimidazole

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Hypertension is the leading risk factor for hu-man morbidity and mortality. However, despite the large number of antihypertensive agents, only a few patients achieve to control their blood pressure. Angiotensin II (Ang II) AT1 receptor antagonists are used successfully in the treatment of hypertension and maintenance of homeostasis. Herein, we describe an efficient synthesis and biological evaluation of AT1 Ang II receptor antagonists based on 4(5)-butylimidazole. These analogues are characterized by different orientation in the substituents of the heterocyclic ring compared to losartan. Additionally, we selected the most potent analogue *1* (2) and the parent compounds *5* and *6* were synthesized through oxidation reactions as its potential active metabolites analogous to EXP 3174. *In vitro* biological evaluation showed high antihypertensive activity for analogues *1* and *6* (pA_2 = 7.97, 7.83, respectively) comparable to losartan (pA_2 = 8.16). This suggests that reorientation of the substituents on the imidazole ring retained activity. On the other hand, a carboxy group at the 2-position of the imidazole ring provides a crucial acidic or hydrogen-bonding point of attachment to the AT1 receptor, in contrast to the absence of a small sized group which is capable of forming hydrogen bond (*7*, pA_2 = 6.2) (3).

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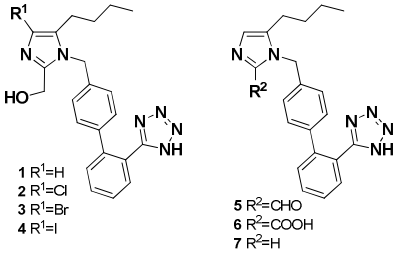

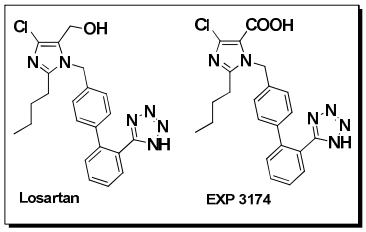

Conformational Elucidation of Aliskiren, a Potent Renin Inhibitor

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Aliskiren is a non-peptidic molecule that specifically inhibits human renin. It has been newly introduced to the pharmaceutical industry as a standalone antihypertensive drug with various effects, especially blockade of the renin receptor. In previous studies, 1D NMR experiments have been performed on aliskiren, but no data is available in terms of its structural characteristics in solution. In this report, the conformational behavior of aliskiren is studied in water (H_2O) and *N,N*-dimethylformamide (DMF) solutions, through dynamic HR 600MHz NMR spectroscopy, by means of 2D-NMR spectroscopy and Molecular Modeling techniques. These results are used to compare the conformation of aliskiren in different solutions with the aliskiren-renin crystallized structure, in order to examine the structural characteristics of this antihypertensive agent. Data extracted on the interatomic distances between distant protons of the molecule's functional groups are combined with molecular dynamics simulations for inspection and evaluation of the drug's molecular properties. Results indicate that the two different solvents impose a *curved* structure in both cases but a different orientation to the pharmacophoric groups.

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Ex vivo Detailed Pathophysiological Assessment of Cardiac Performance *via* High Resolution, High Definition CCD Analysis, of the Isolated Heart

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Cardiac performance measurements on laboratory basis are essential for the evaluation of new pharmaceutical heart treatments. They are mainly based on measurements of LV anatomical changes (Echo, CT, MRI) and heart's pressure changes (Echo, pressure sensors). In order to obtain more detailed cardiac physiological data-for electrical and optical mapping purposes, CCD cameras have been used in connection with voltage sensitive dyes. However these are experimental techniques that demand a high element of expertise and expensive equipment. We developed a new easy and fast CCD analysis tool of the isolated beating heart and aorta. It is based on commercial fast CCD camera technology, home-made software, and real time image acquisition-analysis methods. Its significance relies on the facts that it allows both non-contact and simultaneous examination of all cardiac parameters mentioned above. A high resolution, high definition CCD camera is used to acquire images from the isolated beating heart. Subsequently Image analysis software is used to eliminate noise and provide real time (RT) morpho-metric data (heart chamber's and aortic dimensions and movements) which are transformed to cardiac indexes in order to supply also RT continuous myocardial contractility, heart rate, electrical spreading and regional dynamics numerical data.

Interaction of New Fluoroketolide Antibiotic with the Bacterial Ribosome

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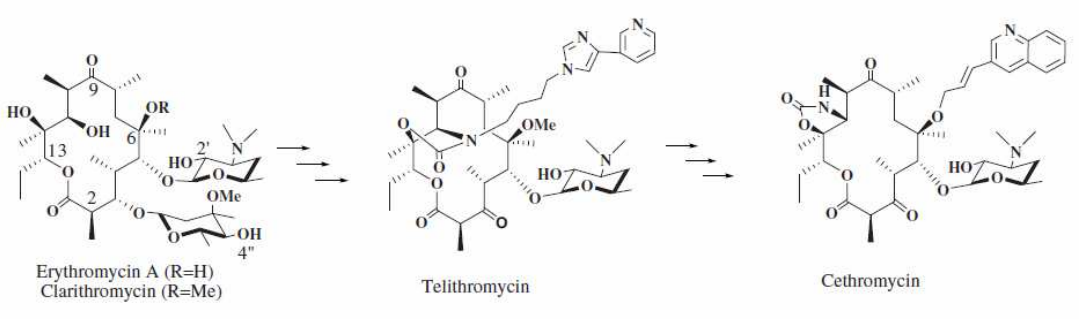
In an effort to combat antibiotic resistance, a newer class of macrolides, known as ketolides, was developed. Ketolides show improved activity against strains with inducible erm genes and are believed to exhibit a tighter binding to the ribosome compared with the previous classes of macrolides. Here we present data concerning some novel promising drugs of this class named fluoroketolides. Their structure is almost similar to that of cethromycin, another important ketolide been already in the last clinical tests, plus the presence of one or two fluoride atoms at C-2 or C-13 of the lactone ring. In microbiological tests involving a number of wild and mutant clinical pathogens, our drugs characterized by lower MIC values than those of erythromycin and telithromycin. *In vitro* studies using a coupled transcription/translation system confirmed the previous data as well as competition experiments with [14C]erythromycin. Chemical protection experiments in domain V of 23S rRNA revealed that all fluioroketolides protected strongly nucleotides A2058, A2059 from dimethyl sulfate modification. This footprint pattern, suggests that all new antibiotics occupy the classical macrolide site at the entrance of the exit tunnel, but the exact position of the alkyl-aryl side chain or additional interacttions concerning fluorine atoms remain to be cleared. Understanding how the side chain and fluorine atoms of these novel ketolides interact with the ribosome will help to guide the future research and development of this important class of antibiotics.



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Differential Antioxidant and Anti-Amyloid Effects of a Wild Blueberry Polyphenol-Rich Extract: *In vitro* and *in vivo* Studies

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In this study, the effects of a daily, 7-day, intraperitoneal (i.p.) administration of a polyphenol-rich extract (PrB) of *Vaccinium angustifolium* (wild blueberries), on cognitive functions were examined in healthy adult (4 months old), male *Balb-c* mice (n=8/group) by passive avoidance test. Whole brain homogenates were collected for examination of brain oxidative markers, caspase-3 and acetylcholinesterase (AChE) activity. Results showed that PrB-treated mice exhibited a significant improvement in learning and memory, accompanied by reduced lipid peroxidation products, higher total brain antioxidant activity and reduced caspase-3 activity. Furthermore, salt- and detergent-soluble AChE activity was signify-cantly decreased. To further delineate the neuroprotective mechanisms, we investigated the antioxidant effects of PrB in three different cell lines against H_2O_2-induced oxidative damage. PrB suppresses H_2O_2-initiated SH-SY5Y intracellular cell death (MTT assay) and oxidation (DCF assay). Moderate effects were observed on CHO^{APP770} cells, while further damage on HEK293 cells was shown after co-treatment with 250 μM H_2O_2 and PrB. Since Amyloid Precursor Protein (APP) altered metabolism, Aβ-overproduction/aggregation are key pathological hallmarks in AD, we further studied the effects of PrB on Aβ-fibrillogenesis, in vitro, utilizing the thioflavine T assay, and on APP-metabolism in CHO^{APP770} cells. Blueberry polyphenols inhibited Aβ-aggregation in a time-dependent manner, while in CHO^{APP770} cells, no alterations in APP metabolism were observed as assessed by western blot. Taken together our results suggest that blueberry polyphenols exhibit antioxidant and/or pro-oxidant properties, according to the cellular environment, inhibit Aβ-fibrillogenesis *in vitro* but have no effect on APP metabolism.

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In Silico Screening towards Novel Trypanothione Synthetase Inhibitors

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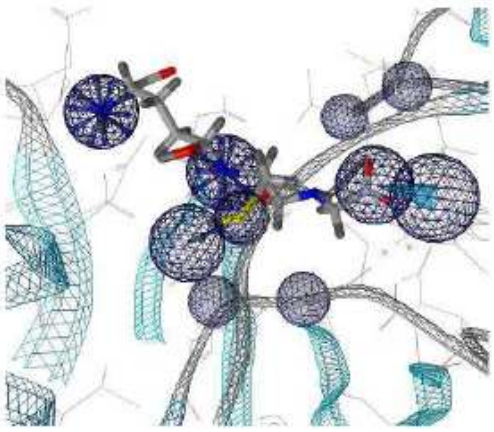

Trypanothinone Synthetase (TryS) is the sole enzyme responsible for the biosynthesis of trypanothione in the human pathogenic parasites *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania major.* Therefore, TryS inhibition is considered to be a particularly attractive strategy to fight leishmanial infections. A model of Leishmania major-TryS has been produced, based on the crystal structures of Leishmania major – Trypanothione Synthetase Amidase (TSA) (1) and E. coli - glutathionylspermidine synthetase (GSPS). Based on the docked complex of the TryS with glutathione, a pharmacophore model was generated. Pharmacophore-based *in silico* screening of commercially available compound libraries in combination with molecular docking studies has led to the identification of putative inhibitor candidates.

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Mechanisms of Non-Fatal Stent Related Myocardial Infarction Late Following Coronary Stenting with DES and BMS: Insights from Optical Coherence Tomography

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The mechanisms of infarction, that are attributed to delayed stent thrombosis, are not studied sufficiently. This is a prospect study of patients with previous angioplasty with stent implantation and new myocardial infarction, between October 2008 and May 2010. In case the infarction was attributed angiographicly (and based on ECG abnormalities in case of infarction with ST segment elevation - STEMI) to a previously implanted stent, the culprit lesion was studied with OCT. We included 17 patients aged 61.7± 9.4 years with median interval between the initial angioplasty and infarction 36 months. From the 17 *culprit* stents, 3 were not analysable due to poor image quality. The medium thickness of neointimal hyperplasia (NIH), were 234.9±227.92 mm, the medium of stent eccentricity index (SEI) was 0.93±0.027 and the medium neointimal unevenness score (NUS) was 1.7±0.36. The total number of uncovered struts was 294 (15.4%). Total number of malapposed struts was 196 (10.1%), with 78 (39.8%) of which were simultaneously uncovered. The total number of protruding struts was 39 (2%), 15 (38.5%) of which were simultaneously uncovered. Thrombus was observed in 10 (71.4%) stents (white clot in 10 and red in 5), necrotic core in 3 (21.4%), neovascularization in 5 (35.7%), and rupture of hyperplastic tissue in 8 (57.1%) with presence of cavity in 7 (87.5%) of these. The delayed stent thrombosis, constitutes an important mechanism of myocardial infarction both in DES and BMS. The presence of necrotic core, neovascularization, and rupture of hyperplastic tissue with cavity formation and thrombus inside the *culprit* stent, suggests a mechanism not different from the rupture of classic atheromatous plaque. Also, the high ratio of uncovered and malapposed struts underlines the need for optimal stent positioning during angioplasty.

A Simple Analytical Method for Aqueous Humour Determination of Topically Administrated Antibiotic Ofloxacin

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Purpose: To determine aqueous humour concentrations after topical administration of 0.3% ofloxacin in different dosing regimens before phacoemulsification surgery using UPLC/MS.
Patients and Method: Patients were randomly selected to receive ofloxacin 0.3% four times/day for one day prior to surgery (Group 1) or four drops 15 min apart (Group 2) 1 h prior to the surgery. At the beginning of surgery, 0.1 ml of aqueous humour was aspirated and the concentrations of ofloxacin were analyzed by Ultra-performance liquid chromatography combined with mass spectrometry. The linearity was obtained in the concentration range of 1.5-3.5 μg/ml for ofloxacin using an internal standard method. The square of the correlation coefficient was 0.999.
Results: The mean concentration of ofloxacin in the aqueous humour was 0.920±0.126 μg/ml in Group 1 and 1.035±0.11 μg/ml, in Group 2.
Conclusion: This analytical method is simple, fast, linear and precise for the determination of ofloxacin in aqueous humour. Ofloxacin penetrated the aqueous humour equally in both dosing regimens during cataract surgery and reached and exceeded the minimum inhibitory concentration levels for the most common ocular pathogens causing endophthalmitis.

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A New Method for the Determination of Free and Total L-Carnitine in Serum Samples Based on ^{1}H-NMR Spectroscopy

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The rapid and accurate determination of specific metabolites present in biofluids is a very demanding task which is essential in both medicine and chemistry. L-carnitine (3-hydroxy-4-N-trimethylammonium butyrate) is an important metabolite which participates in a series of biological paths and therefore its determination is of diagnostic importance. A single quantum coherence filtering ^{1}H NMR methodology was used for the accurate and rapid determination of L-carnitine in human serum samples. The methodology is based on spectral simplification, and specifically on the distinction of the N-methyl proton signal of L-carnitine that is greatly overlapped in the ^{1}H-NMR spectrum of serum. The quantitative results provided by the proposed method are in excellent agreement with those obtained by the enzymatic method, which is widely used. The proposed method is rapid, selective, sensitive, and has good analytical characteristics (accuracy, reproducibility).