Review of Clinical Pharmacology and Pharmacokinetics

EPITHEORESE KLINIKES FARMAKOLOGIAS KAI FARMAKOKINETIKES
ΕΠΙΘΕΩΡΗΣΗ ΚΛΙΝΙΚΗΣ ΦΑΡΜΑΚΟΛΟΓΙΑΣ ΚΑΙ ΦΑΡΜΑΚΟΚΙΝΗΤΙΚΗΣ
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Drug Discovery and Design

Guest Editor
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Head of Medicinal Chemistry Postgraduate Program
Medicinal Chemistry: Drug Discovery and Design

Letter from Guest Editor

This issue contains Abstracts of research work presented by specialists at the Sixth Medicinal Chemistry Conference held at the University of Patras, 10-12, March 2005. This Conference was organized by the Postgraduate EPEAEK Program *Medicinal Chemistry: Drug Discovery and Design* initiated and sponsored by the Ministry of National Education and Religion.

This Program is offered by the Departments of Chemistry and Pharmacy of the University of Patras, to selected graduate students from Departments of Chemistry, Pharmacy, Biology and Medicine. In particular, this issue contains articles, which are the results of novel work carried out by the researchers of the program and their graduate students, who take the post graduate program leading to Master of Science and PhD degrees. Abstracts cite in summary research findings from a broad area of Biomedical Fields, including Organic Synthesis, Biochemistry, Biomedical Analysis, Molecular Modeling, Pharmacology and Drug Design Methods. The articles of the book are written by specialists in their field, who participated at the Conference and provide a global understanding of the recent activities in the field of Drug Discovery and Design in Greece and Abroad.

The Guest Editor, on behalf of the Postgratuate Program Committee, wishes to express his deep appreciation to all contributors in this book. We also thank the Editorial Board of Review of Clinical Pharmacology and Pharmacokinetics in particular Journal Editors S. Plessas and C. Plessas for invitation and for providing the suitable and high-standard forum through which important findings of this research will become available to the scientific community.

The Guest Editor

John Matsoukas

Professor in Chemistry
University of Patras, Greece
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Medicinal Chemistry: Drug Discovery and Design

REVIEW OF CLINICAL PHARMACOLOGY AND
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Singlet Oxygen: Toxicity and Therapeutic Properties

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Key words: Singlet oxygen, toxicity, therapeutic properties

The organic, biological and medicinal chemistry of molecular oxygen, in its ground and excited (Δg, $^1O_2$) states, is of extraordinary interest (1-4). Oxygen plays an important role in aging, damage to materials in the environment, cellular pathology (for example the damage following stroke or heart attack) and in many other areas (5). In this presentation, a short overview of the key issues of sensitized photooxidations in organic biological and medicinal systems will be attempted.

$$\text{Sens} \xrightarrow{h\nu} {}^*\text{Sens} \xrightarrow{O_2} {}^1O_2$$

REFERENCES

1. Singlet Oxygen: Wasserman, H.H.; Murray, R.W., Eds. Academic Press, New York, 1979
2. Straight R.C., Spikes J.D.: In: Singlet O_2 (Frimer A.A., ed.). Pp. 85-144, CRC Press, Boca Raton FL, 1985
3. Stratakis M., Orfanopoulos M.: *Tetrahedro 56*: 1595-1615 (2000) Review.
4. Adam W., Bottke N., Krebs O., Lykakis I., Orfanopoulos M., Stratakis M.: *J. Am. Chem. Soc. 124*: 14403-14409 (2002)
5. *Med. Ad. News vol. 21*, number 5, page 48. May 2002

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New Opportunities for the Treatment of Pain and Inflammation: The Endocannabinoid System and Inhibitors of Phospholipase A_2

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Key words: Pan, inflammation, endocannabinoid system, phospholipase A_2 inhibitors

The interest in new treatments for pain and inflammation has been recently increased, because current therapies have been associated with undesirable side effects and, in various cases (e.g. neuropathic pain), have been proved unsuccessful. This presentation

will focus on the opportunities provided by the endocannabinoid system and inhibitors of phospholipase A_2 (PLA_2) for the development of new medications for pain and inflammation. Five proteins involved in the endocannabinoid system, namely CB_1 and CB_2 receptors, anandamide transporter, fatty acid amide hydrolase (FAAH) and monoglyceride lipase (MGL) are excellent potential targets. In our lab we have designed and synthesized the first inhibitors of MGL. On the other hand, we have designed and synthesized novel inhibitors of human GIVA PLA_2, which may regulate the production of arachidonic acid and PGE_2 in cells and demonstrate potent *in vivo* anti-inflammatory and analgesic activities.

Financial support from *PYTHAGORAS* program is gratefully acknowledged.

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Apolipoprotein A-I Peptide Models as Probes for Developing Atheroprotective Agents

Maria Sakarellos-Daitsiotis, Charis Alexopoulos, Maria Petraki, Afroditi Tambaki, Konstantinos Harilogis, Alexandros Tselepis and Constantinos Sakarellos

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Key words: Apolipoprotein A-I, peptide models, atheroprotective agents

The inverse relationship between plasma levels of high density lipoprotein (HDL) and the risk of atherosclerosis and coronary artery disease has been attributed to several HDL functions, including antioxidant and antiinflammatory effects. An important role in these effects plays the apolipoprotein content of HDL, especially apolipoprotein A-I (apoA-I). With the aim to develop atheroprotective agents, we report on the design, synthesis, conformational analysis and biological effects of four amphipathic α-helix apoA-I peptide models:

Ac-ESK(Palm)KELSKSW^{10}SEM^{13}LKEK(Palm)SKS-NH_2 (1),
Ac-ESK(Palm)KELSKSM^{10}SEW^{13}LKEK(Palm)SKS-NH_2 (2),
Ac-FKEFSKSMSEWFKEF-NH_2 (3),
Ac-FKEFSKSASEWFKEF-NH_2 (4)

where Glu and Lys residues constitute the hydrophilic face, while Met, Phe, Leu, Trp as well as Palmitoyl-groups the spatially segregated hydrophobic phase of the amphipathic α-helix. Met could serve as additional oxidant-scavenger for protecting LDL from irreversible oxidative damage and Trp as intrinsic fluorescence probe. The syntheses of the apoA-I peptide models were carried out following the Fmoc-strategy and an orthogonal protection system. The helical characteristics of the apoA-I peptide models in their reconstituted form in POPC, DMPC, DMPG were studied by CD spectroscopy. The ability of the apoA-I

peptide models to inhibit Cu^{2+}-induced oxidation of the low-density lipoprotein (LDL) *in vitro*, and to prevent the oxidation induced inactivation of the LDL-associated platelet-activating factor acetylhydrolase (PAF-AH) at different concentrations were investigated and their atheroprotective role is discussed.

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Efficient Syntheses of Polyaminotetrazoles as Lead Compounds for the Development of Polyamine-Based Pharmaceuticals

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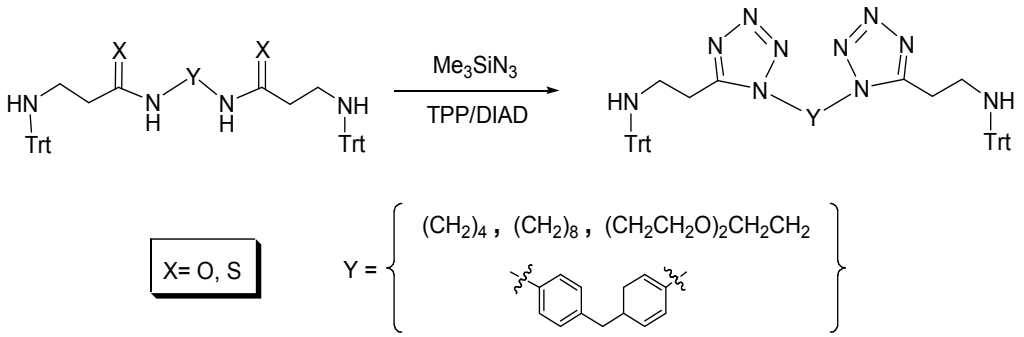
Key words: Polyaminotetrazoles, synthesis, polyamine-based pharmaceuticals



5-Substituted-1*H*-tetrazoles and 1,5-disubstituted-tetrazoles are often used as metabolism-resistant isosteric replacements for carboxylic acids and as *cis* amide bond surrogates, respectively, in SAR-driven analog synthesis in medicinal chemistry (1-3). Although a variety of synthetic tetrazole-containing biologically active substances are described in the current literature there are no examples of polyamine-based tetrazoles. We have developed a general methodology that provides easy access to linear 5-aminoalkyl-1*H*-tetrazoles and polyamines incorporating tetrazole units in their skeleton, by activating, where necessary, secondary amide bonds through thionation toward their reaction with azidotrimethylsilane under Mitsunobu reaction conditions (4).

REFERENCES

1. Herr R.J.: *Bioorg. Med. Chem. 10*: 3379-3393 (2002) and references cited therein.

2. Yu K.L., Johnson R.L.: *J. Org. Chem. 52*: 2051-2059 (1987)
3. Zabrocki J., Smith D., Dunbar J.B.Jr., Iijima H., Marshall G.R.: *J. Am. Chem. Soc. 110*: 5875-5880 (1988)
4. Athanassopoulos C.M., Garnelis T., Vahliotis D., Papaioannou D.*: Org. Lett.* 2005, in press.

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Pharmacokinetic Studies in Rodents for the Discovery of New Drugs by Liquid Chromatography Tandem Mass Spectrometry (Lc-Ms-Ms)

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Key words: New drugs, pharmacokinetics studies, Lc-Mc-Ms, rodents

A typical medicinal chemistry program generates numerous new leads that require further characterization in order to guide investigators (chemists) towards the synthesis of analogues that are more *drug like*. One of our main objectives is to aid medicinal chemists through our expertise in state of the art bioanalytical techniques such as tandem mass spectrometry coupled with pilot *in vivo* studies in rodents. Rapid answers can be obtained to questions of bioavailability, plasma clearance, brain penetration and overall tissue distribution, all critical components of the drug discovery process. We have elected an example of a potent cyclic peptide in order to demonstrate how LC-MS-MS can be employed towards sensitive measurements of peptide in rat plasma and how an extension of the approach allows the measurement of peptide in rat brain following intravenous administration. The type of information obtained from those studies is crucial for the *in vivo* pharmacologic evaluation of new chemical entities and can lead to the design of molecules with improved pharmacokinetic properties.

REFERENCES

1. Mock S., Shen X., and Tamvakopoulos C.: *Rapid Communications in Mass Spectrometry 22*: 2142-2147 (2002)
2. Trivedi P., Jiang M., Tamvakopoulos C., Shen X., Hong Yu, Mock S., Fenyk-Melody J., Van der Ploeg L.H.T. and Guan X.: *Brain Research 977*: 221-230 (2003)

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Ground-Hemlock, *Taxus Canadensis*, as a Source of Paclitaxel

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Key words: *Taxus Canadensis*, paclitaxel

Paclitaxel, a naturally derived anti-cancer drug, was discovered by scientists at the National Cancer Institute (NCI) in the 1960's. By 1983 the first human trials of the compound's antitumour activity began and in 1991 Bristol-Myers Squibb (BMS) secured the rights from NCI to produce and develop the drug.

The original source of paclitaxel was the bark of the pacific yew (*Taxus brevifolia),* which was limited in availability and unlikely to meet the projected needs for the drug which was approved for ovarian and breast cancer therapy.

Attention turned to the common ornamental plant, the English yew (*Taxus bacccata*) from which a compound, 10-deacetylbaccatin III or 10-DAB, a close chemical relative of paclitaxel, could be obtained and was convertible by semi-synthesis, on a commercial basis, to the anti-cancer agent.

In the early 1990's interest in finding other sources of paclitaxel (value ~US $600,000 per kg, at that time) rapidly developed and it was soon learned that ground hemlock (*Taxus canadensis*), a common shrub in the eastern North American forests, contained substantial quantities of paclitaxel as well as 10-DAB, however the major "taxane" component of ground-hemlock proved to be another close relative of paclitaxel namely 13-acetyl-9-dihydrobaccatin III or DHB.

An efficient, three-step chemical transformation of the relatively abundant 13-acetyl-9-dihydrobaccatin III (DHB) into 10-deacetylbaccatin III (10-DAB), the established paclitaxel semi-synthesis precursor, will be presented.

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Research and Development of Anticancer Drugs

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Key words: Anticancer drugs, R&D

In the last decades our knowledge has greatly increased in regard to the structure, synthesis and mechanism of action of compounds with anticancer activity. Research and

development of anticancer drugs is a lengthy process, taking over 12 years and requiring hundreds of millions Euros, from the identification of a suitable drug target to the introduction of a new medicine. Following resolution of intellectual property issues and successful initial *in vitro* experiments, a new compound undergoes *in vitro* toxicity screening on a variety of tumour cell lines. Only 2% of the tested entities continue to *in vivo* animal experiments, where the biodistribution, toxicity and efficacy are determined. Only 1-2 out of 10,000 new molecules successfully complete this stage of development and are considered for testing in humans. Phase I and II clinical trials are conducted with a limited number of patients to determine safety, dosage and effectiveness. Following this, Phase III studies are initiated, with larger number of patients typically in a multicentre/multinational setting, where the safety is confirmed and the efficacy of the new treatment is determined as compared with existing therapies. Only 20% of the new compounds, which enter clinical development, successfully reach the market, where research continuous through Phase IV clinical trials and pharmacovigillance procedures.

REFERENCES

http://cancer.gov/http://www.nih.gov/
http://www.fda.gov/ ΦΕΚ ΔΥΓ3/89292/31-12-2003

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Role of Harp on the Growth of Glioblastoma Cells

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Key words: Glioblastoma cells, harp

Heparin affin regulatory peptide (HARP) seems to be involved in the progression of brain tumors, such as the glioblastoma multiforme, with mechanisms yet to be elucidated. Glioblastoma multiforme is the most aggressive malignant glioma and among the highly angiogenesis-dependent tumors. In this study, we tried to determine the role of HARP in glioblastoma multiforme, using antisense strategy to inhibit the endogenously expressed HARP in C6 glioma cells. Reduction of HARP protein in C6 cells affected proliferation rate and anchorage-independent growth of these cells, increased tumor-induced angiogenesis *in vitro* and *in vivo* and vascular endothelial growth factor (VEGF) mRNA levels. Conclusively, HARP seems to have a significant effect on tumor growth and angiogenesis induced by C6 glioma cells.

We thank European Social Fund (ESF), Operational Program for Educational and Vocational Training II (EPEAEK II), and particularly the Program IRAKLEITOS, for funding the above work.

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Inhibition of Tumor Cell Growth with Therapies Using Small Molecules

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Key words: Inhibition tumor cell growth, small molecules, therapies

Over the recent years therapy in oncology has been dominated by advances in the field of pharmacogenomics. In its broadest definition, pharmacogenomics encompass studies of the genotype and phenotype of tumor cells that can predict the likelihood of response to a specific treatment. This treatment modality is called targeted therapy and includes not only chemotherapy treatments but also new type of treatments such as monoclonal antibodies and small molecules.

One of the first type of small molecule based treatment in oncology was the development of hormone therapies for patients with breast and prostate cancer. The drug tamoxifen is the most widely prescribed antiestrogen for the treatment of women with breast cancer expressing estrogen and progesterone receptors since 1970.During the last years additional biomarkers have been used to further refine hormone therapy responses that supported the introduction of new hormone treatments such as aromatase inhibitors

All-Trans Retinoic Acid (ATRA) has shown significant clinical benefit for patients with acute promyelocytic leukaemia that feature t(15:17) a disease defining translocation. Direct targeting of the retinoic acid receptor with ATRA results in high overall disease response rates, delay in disease progression, and long-term cures.

The introduction of Imatinib Mesylate (Gleevec) for patients with chronic myeloid leukaemia bearing the bcr/abl translocation was accompanied by a lot of excitement by the scientific and public communities for the future potential of low-toxicity, targeted anticancer therapy. Treatment with imatinib, an adenosine triphosphate–binding selective inhibitor of bcr-abl, has been associated with durable complete hematologic and complete cytogenetic remissions with minimal toxic effects in the early chronic phase of CML. Imatinib has also a role for the treatment of relapsed and metastatic gastrointestinal stromal tumors (GISTs), which characteristically bear an activating mutation in the c-kit receptor tyrosine kinase (RTK) gene.

Gefitinib was recently approved as monotherapy for patients with inoperable non–small cell lung cancer that fail to respond to chemotherapy treatment. Gefitinib is a small-molecule drug that targets epidermal growth factor receptor (EGFR) and is easily identified by immunohistochemical analysis. This receptor is found in lung and other cancers but multiple cellular biochemical pathways are involved in its regulatory function limiting the use of gefitinib to specific tumor phenotypes.

Erlotinib is a novel very promising inhibitor of EGFR in late-stage clinical trials for the treatment of non–small cell lung cancer, pancreatic cancer, and primary glioma.

Thalidomide, Endostatin, and Angiostatin) are small-molecule drugs that target tumor blood vessels (antiangiogenesis drugs) are in clinical trials for a variety of malignant neoplasms. Until today, the development of a test

(such as tumor microvessel density or the expression of an angiogenesis-promoting gene or protein) for the identification of patients that benefit more from this type of treatment has proved a difficult task. Nevertheless, its use in patients with refractory multiple myeloma has shown enormous clinical benefit.

Recently, drugs targeting the proteasome have been developed that are designed to impact downstream pathways regulating angiogenesis, tumor growth, cell adhesion, and resistance to apoptosis. One of these agents, bortezomib, was approved in May 2004 for the treatment of relapsed and refractory multiple myeloma. Bortezomib inhibits the nuclear factor kB (NFkB) and is under investigation for the treatment of early-stage multiple myeloma, non-Hodgkin lymphoma, and a variety of solid tumors, including lung cancer.

During the next several years, the field of oncology drug development will see numerous products pass through the approval process and enter the market accompanied by diagnostic tests designed to *personalize* their use, dosage, route of administration, and length of treatment for each patient, one at a time. Only time will tell whether this new approach to anticancer pharmaceuticals will yield breakthrough results, reducing morbidity and mortality and improving outcomes for all who will be afflicted with the disease.

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Inhibition of Cancer with Monoclonal Antibodies

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Key words: Monoclonal antibodies, cancer, inhibition

Monoclonal antibodies (MAbs) are capable of targeting certain antigens with remarkable specificity such as tumor associated antigens, growth factor receptors etc having the potential to destroy malignant cells by immunological mechanisms. MAbs have been used to treat a number of common malignancies either reacting with growth factor receptors (HER-2, $EGFR_{1, ...}$) or as active immunogens, activating cellular effector mechanisms (ADCC, complement activation, etc) or as carriers of toxins, drugs, enzymes, photosensitizers and radioisotopes. Preclinical as well as clinical studies with MAbs have led to some striking examples of antitumor effects. However, the majority of early trials served primarily to illustrate the obstacles to successful therapy, particularly when using unconjugated MAbs that are designed to stimulate and focus host immune responses on tumor sites. Current clinical data confirm that immunotherapy with MAbs is an effective treatment in a number of common malignancies including breast, colorectal and head and neck cancer, NHL and leukemias resulting in a significant reduction in mortality and tumor recurrence rates. Immunotherapy of certain tumors with MAbs has currently incorporated chemotherapy in order to improve clinical results either by eliminating residual drug-resistant tumor cells or sensitizing tumor cells to chemotherapy-induced apoptosis. Additional

studies are required in order to optimize the methods for enhanced antibody targeting in tumors, to reduce serious adverse events and identify subgroups in patients who might benefit more from immunotherapy with MAbs. The development of new molecules such as immune fragments, peptides, cancer specific and chimeric/humanized MAbs as well as anti-idiotypic antibodies is a major challenge currently in terms on how to integrate these new treatments to existing molecules. In conclusion, immunotherapy with MAbs represents an established new therapeutic anticancer modality. In the future, MAbs will have a far more increased impact on the management of patients with cancer.

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Safety and Immunogenicity of the Optimized Cryptic Peptide $TERT_{572Y}$ in Patients with Advanced Malignancies: A Phase I Clinical Study

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Key words: Optimized cryptic peptide $TERT_{572Y,}$ patients with advanced malignancies, phase I clinical study, safety, immunogenicity

Background: $TERT_{572Y}$, an optimized cryptic peptide homologous to TERT induces efficient antitumoral T cell cytotoxic immunity but not autoreactivity *in vivo* in HLA-A*0201 transgenic mice and healthy blood donors and prostate cancer patients (J. Clin. Invest., 2004, 113,425). A phase I trial evaluating the safety and immunogenicity of the cryptic $TERT_{572Y}$ peptide was conducted in HLA-A*0201 cancer patients.

Patients and methods: Nineteen patients with chemotherapy refractory and progressing malignant tumors were enrolled in the study. The vaccination protocol consisted of two injections of optimized $TERT_{572Y}$ peptide followed by four injections of native $TERT_{572}$ peptide. Peptides were injected emulsified in Montanide ISA51. Patients were vaccinated with escalated doses of peptide ranging from 2 to 6 mg. Toxicity and peptide-specific immune responses were evaluated.

Results: Fourteen patients completed the entire vaccination program. Only grade I toxicity was observed, affecting 13 of the 19 patients. $TERT_{572Y}$-specific cytotoxic T cells were detected in the peripheral blood of 13 out of 14 evaluate patients, as early as 3 weeks after the 2^{nd} vaccine injection. CTLs were fully functional and killed TERT-overexpressing tumor cells. Four (29%) of 14 evaluable patients experienced stable disease for a median of 10 months.

Conclusion: $TERT_{572Y}$ peptide vaccine is well tolerated and effective in eliciting a specific T cell immunity. This is the first clinical trial demonstrating that cryptic peptides are promising candidates for cancer immunotherapy.

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Human Autoimmune Disorders and Chronic Rejection of Organ Allografts are T-Cell Diseases Driven by Specific Antigens

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Key words: Human autoimmune disorders, organ allografts, T-cell diseases, specific antigens

We have shown that major autoimmune diseases and chronic rejection of organ allografts in humans are specific antigen-driven T-cell diseases. Our research group has used a combination of molecular and cellular approaches to reach these conclusions. These approaches include the examination of appropriate target tissues for: (i) the presence of infiltrating mononuclear cells (CD3-positive T cells) expressing early, intermediate and late activation antigens; (ii) the expression of T-cell derived cytokines (identified using DNA microarrays and other approaches); (iii) the presence of monoclonal/oligoclonal populations of alpha/beta T-cell receptor (TCR)-positive and gamma/delta TCR-positive T cells, identified by the presence of multiple identical copies of TCR transcripts in appropriate target tissues; and (iv) other evidence. Strong evidence suggest that: (i) osteoarthritis (OA) (in approximately 40-50% of the patients examined); (ii) systemic sclerosis (SSc); (iii) abdominal aortic aneurysms (AAA); (iv) demyelinating disease of the CNS; and (v) chronic rejection of cardiac allografts; are specific-antigen driven T-cell diseases and that T cells play a central role in their pathogenesis. Additionally, the immune response of the host to the tumor exhibits similar characteristics. Clonally expanded T cells in these diseases are of functional significance and very likely responsible at least in part for the initiation and/or the propagation of these autoimmune disorders, the rejection of organ grafts and the immune response of the host to the tumor. T cells provide the “engine” for the development and maintenance of chronic inflammation. Molecular mimicry is one of the possible mechanisms that may be involved. The identification of specific antigen-driven T-cell responses and of clonally expanded *in vivo* TCR transcripts in these diseases will permit the identification of specific antigens that elicit these responses and may be are responsible for the pathogenesis of these diseases. These results substantially improve our understanding of these disorders for which effective treatment is not available. A

number of these disorders need to be reclassified and new molecular and cellular approaches need to be developed incorporating our new understanding of these diseases to achieve more effective treatment protocols.

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Neurosteroids as Neuroprotectants

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Key words: Neurosteroids, neuroprotectants

The neuroactive steroids dehydroepiandrosterone (DHEA), its sulfate ester DHEAS and allopregnanolone (Allo), produced by the central nervous system and the adrenals, appear to exert a protective effect in hippocampal and cortical neuron ischemia- and excitotoxicity-induced injury (1,2). These steroids they may also play a protective role on the adrenal medulla, an important part of the sympathetic nervous system, and the tissue adjacent to their primary site of production. Indeed, DHEA, DHEAS and Allo protect rat chromaffin cells and the rat pheochromocytoma PC12 cell line, an established model for the study of adrenomedullary cell apoptosis and survival, against serum deprivation-induced apoptosis. Their effects are time- and dose-dependent with EC_{50} 1.8, 1.1 and 1.5 nM respectively (3). The anti-apoptotic effect of DHEA(S) and Allo is found to be structure-specific, confined mainly to conformation 3beta-OH-Δ^5 for androstenes and 3alpha-OH for pregnanes. Indeed, 3-keto, Δ^4, or C7 hydroxylated androstenes and 3beta pregnanes are ineffective. The prosurvival effect of DHEA(S) appears to be NMDA-, $GABA_A$-sigma1- or estrogen receptor independent, and is probably mediated by a specific G-protein coupled membrane receptor. It involves the anti-apoptotic Bcl-2 proteins, their role being sine-qua-non for their action since Bcl-2 antisense oligonucleotides reverse their effects. Furthermore, DHEA(S) and Allo activate CREB and NF-κB, up-stream effectors of the anti-apoptotic Bcl-2 proteins expression. They also activate most prominent pro-survival kinases, such as PKCα/β, MEK/ERK and PI3K/Akt. Our findings suggest that decline of DHEA(S) and Allo during ageing or stress may leave the adrenal medulla unprotected against proapoptotic challenges.

REFERENCES

1. Baulieu E.E., Robel P.: *Proc. Natl. Acad. Sci. USA 95*: 4089–4091 (1998)

2. Compagnone N.A., Mellon S.H.: *Front Neuroendocrinol. 21*: 1-56 (2000)

3. Charalampopoulos I., Tsatsanis C., Dermitzaki E., Alexaki I., Castanas E., Margioris A.N., Gravanis A.: *Proc. Natl. Acad. Sci. USA 101*: 8209-8214 (2004)

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Identification of Antigen(S) in Demyelinating Disease through Molecular Analysis of the T-Cell Receptor

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Key words: Demyelinating disease, antigen(S), T-cell receptor, molecular analysis

MS is an inflammatory demyelinating disease that may be initiated in genetically susceptible individuals by an as yet unidentified virus aquired at childhood. About 85% of the MS cases manifest as classical chronic remitting-relapsing and secondary progressive autoimmune demyelinating disease of the CNS. Clonally expanded T cells have been reported in the CNS of these patients with chronic MS, however, they may represent epitope spreading because of the chronic nature of these forms of MS. In contrast, short-term or acute MS are more appropriate to study in order to obtain information on T cells that may be involved in initiation or early propagation of the disease. To elucidate the role of T cells in the pathogenesis of the disease, we investigated the clonality of T cells present in the CSF or in brain plaques of patients with acute or short-term MS or MS-like disease. Multiple copies of identical α- and β-chain TCR transcripts were found demonstrating the presence of oligoclonal populations of T cells. To identify putative MS antigens recognized by T cells expanded in vivo, in brain plaques or the CSF of these patients we employed a molecular reconstitution strategy of the clonally expanded TCR. This approach involves the following steps: (1) construction of full-length copies of the clonally expanded α- and β-chain TCR transcripts; (2) expression of the full-length clonally expanded α- and β-chain TCR transcripts into αβ TCR-negative mutant Jurkat T cells using a retroviral vector; (3) determination whether these transduced Jurkat T cells with the clonally expanded TCR recognize, as determined by cytokine production, putative neuroantigen(s) or viral peptides presented by autologous antigen presenting cells (APC). Using the approach described above, we constructed and expressed in mutant Jurkat T cells full-length clonally expanded α- and β-chain TCR transcripts in the CSF of a patient with multiphasic disseminating encephalomyelitis (MS-like demyelinating disease) following infection with hepatitis A virus. These transduced T cells produce cytokines in response to a myelin peptide presented by autologous APC. This is a general approach that can be employed for the identification of putative antigens expressed on appropriate APC to engineered T cells expressing in vivo clonally expanded TCR.

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Cellular Activation and Cytokine Expression Profiles in Autoimmune Diseases and Organ Transplantation: Modulation by Therapeutic Peptides, Sparing the T Regulatory Cells

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Key words: Cellular activation, cytokine expression, autoimmune diseases, organ transplantation, therapeutic peptides, T regulatory cells

In autoimmune diseases, the immune system reacts against autologous antigens and causes cell and tissue damage. Autoimmune diseases are many, and they affect about 3% of the world population. The incidence of autoimmunity depends on genetics, sex and the environment. Today's central idea of autoimmunity involves an immune response of a genetically predisposed individual to an environmental pathogen under the influence of inadequate or non-functional immunoregulatory mechanisms (1-4). Advances in the treatment of autoimmune diseases, follow a better understanding of the abnormalities in the cellular activity pathways and the resulting, and often permanent, imbalance of the pro- and anti-inflammatory cytokine expression profiles (5,6). Over the past few years, there has been a dramatic change in the therapeutic regimens employed in autoimmune diseases, targeting defined pathways of the adaptive immune response. New therapeutic modalities, either in clinical practice or in the experimental stage, include monoclonal antibodies (mAbs), already in use in rheumatoid arthritis patients, vaccines, DNA vaccines and gene therapies. Another approach towards the therapeutic management of autoimmune diseases, involves the design and use of peptide analogs of disease-associated epitopes, to be used as immunomodulatory drugs (7,8). The mechanisms of peptide action that have been proposed so far include: (I) A peptide can act as an antagonist of the wild type antigen for TCR binding, thus interfering with the whole process of antigen presentation and T-cell activation; this can result to apoptosis or anergy of the auto-reactive T-cell clones. (II) A peptide can block or reduce T-cell proliferation and/or (III) induce the polarization of Th0 uncommitted cells to differentiate into anti-inflammatory Th2 cells (immune deviation). Finally, (IV) a peptide can induce peptide-specific regulatory T-cells that cross-react with cognate self-antigen (bystander suppression) (8-11). The peptides can thus arrest the ongoing inflammatory response, interrupt the adaptive memory response in both T- and B-cell compartments, and/or induce antigen-specific immunomodulation and establish tolerance (8-11). Our research group focuses on auto-antigen-specific peptides that meet certain constraints, i.e. they must suppress autoimmune activity

but, also, they must spare the regulatory T-cells (Tregs). Another approach to therapeutic peptides is the design and synthesis of peptide analogs that inhibit the binding of cytokines to their receptors. This approach is not antigen-specific, but aims to suppress the activation of T-cells by any antigen, at a very early stage. In this approach, the same peptides can be used for many disease states in which activated T-cells are major players in their pathology, such αs organtransplantation and certain types of autoimmune diseases. We are currently working towards the development of peptides that can block cytokine binding to their receptor and are suitable for *in vivo* use. As a working model, we use the interleukin 2 (IL-2)/IL-2 receptor (IL-2R) system: Human interleukin-2 (IL-2) is a major regulatory 15.5 kD cytokine (12,13). IL-2 is secreted primarily by activated naive helper T-cells (Th0) and, in turn, it activates helper T-cells (both Th1 and Th2 types), cytotoxic T-cells, B-cells, NK cells and macrophages (14,15). IL-2 binds to and mediates its biological effects through a receptor complex consisting of three distinct subunits (chains) designated IL-2Rβ (CD25), IL-2Rβ(CD122) and IL-2Rγ_c (CD132) (14). The IL-2Rβ and γ_c chains are also utilized by IL-15, a pleiotropic pro-inflammatory cytokine that is implicated in several inflammatory disorders (16). The exclusive expression of the high affinity IL-2R on the surface of activated T-cells makes it an attractive target for the selective inhibition of alloreactive T-cells in organ transplantation or self-reactive T-cells in type-1 autoimmune diseases. In the last 25 years, a lot of research has produced a plethora of anti-IL2R mAbs to be used in the treatment of a wide variety of heterogeneous diseases that have as common denominator high levels of IL-2R expression. The majority of anti-IL-2R mAbs that are used therapeutically in organ transplantation, recognize the α chain (17,18). Fairly recently, it was shown by many research groups that IL-2 promotes the thymic development and peripheral expansion of Tregs, an important population of T-cells that promotes immunological tolerance (19-21). Although Tregs in humans have diverse phenotypes, the majority are CD4CD25 T-cells. Thus, current treatments involving anti-IL-2Rα mAbs could diminish the Tregs/CD4CD25 populations also, leading, in the long term, to the development of severe type-1 autoimmune diseases. Therefore, developing therapeutic strategies that do not involve anti-IL-2Rα mAbs would lead to safer drugs for immunotherapy. To take this point further, replacing mAbs altogether by peptides that block cytokine binding to its receptor, thus inhibiting cell activation without destroying the receptor-bearing cells (through antibody-mediated complement lysis and/or opsonization), is an attractive prospect. Work in our group with peptides designed to map to epitopes of the IL-2Rβ, has produced encouraging preliminary results (22, 23): These particular amino acid sequences are putative IL-2 binding domains because they are located within epitopes recognized by monoclonal antibodies to the IL-2Rβ-subunit, that block binding of IL-2 to its receptor (22). Three of these peptides inhibited mitogen-induced proliferation of peripheral blood T-cells, and two of those had no effect on the percentage of the CD4CD25 T-cells (23). Future experiments will show whether these peptides have *in vivo* activity. In addition, we are currently working on synthetic peptide modifications in order to get molecules that will be stable enough for *in vivo* use.

REFERENCES

1. Ermann J. ad Fathman C.G.: *Nat. Immunol. 2*: 759 (2001)
2. Marrack et al.: *Nat. Med. 7(8)*: 899 (2001)
3. Cooper G.S., and Stroehla B.C.: *Autoim. Rev. 2*: 119 (2003)
4. Kukko et al.: *Diabetes Care 27*: 676 (2004)
5. Mouzaki et al.: *Blood 100*: 1774 (2002)
6. Panitsas et al.: *Blood 103*: 2645 (2004)
7. Hohfeld R.: *Brain 120*: 865 (1007)
8. Mouzaki A., et al.: *Curr. Neurovasc. Res. 1*: 325 (2004)
9. Genain C.P. and Zamvil S.S.: *Nature Med. 6*: 1098 (2000)

10. Nepom G.T.: *Curr. Opin. Immunol. 14*: 812 (2002)
11. Anderton S.M.: *Immunology 104*: 367 (2001)
12. Interleukin-2 (IL-2), IL-2 receptors. In: Immunobiology, the Immune System in Health and Disease (Janaway C.A., Travers P., Walport M., Shlomchik M., eds). 5th edition, Garland Publishing, New York, 2001
13. Smith K.A.: *Curr. Opin. Immunol. 4*: 271 (1992)
14. Nelson B.H. and Willerford D.M.: *Adv. Immunol. 70*: 1 (1998)
15. McDyer et al.: *J. Immunol. 169*: 2736 (2002)
16. Li et al.: *Nat. Med. 7*: 114 (2001)
17. Morris J.C. and Waldman T.A.: *Ann. Rheum. Dis. 59(suppl I)*: i109 (2000)
18. Church A.C.: *QJM 96*: 91 (2003)
19. Ng et al.: *Blood 98*: 2736 (2001)
20. Cosmi et al.: *J. Immunol. 169*: 2736 (2002)
21. Nelson B.H.: *J. Immunol.* 172: 3983 (2004)
22. Dionyssopoulou et al.: *J. Immunol. Methods 24*1: 83 (2000)
23. Parissi et al.: *Transplantat. Proc. 36*: 1723 (2004)

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Animal Models in Multiple Sclerosis: The Use of Myelin Altered Peptide Ligands

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Key words: Multiple sclerosis, myelin altered peptide ligands, animal models

Much of our knowledge regarding the underlying mechanisms in Multiple Sclerosis pathogenesis has been achieved through research on the principal animal model of the disease, the experimental allergic encephalomyelitis (EAE). Like MS, EAE is characterized by $CD4^+$-mediated inflammatory lesions in the central nervous system (CNS) followed by demyelination and axonal damage. Inflammatory infiltrates are consisted of T-cells that are activated against several myelin antigens that are vulnerable in autoimmune attack. Other inflammatory components like cytokines, chemokines, adhesion molecules, macrophages, complement activation and antibodies are also involved in the immune – mediated cascade of myelin destruction. In addition, the mechanism of action of a number of the currently used immunomodulating agents in MS therapy or those tested in ongoing clinical trials were first indicated in EAE. Among them are the altered peptide ligands (APL) which are native peptides modified by amino-acid substitutions at essential contact residues for the TCR. These agents can modulate T-cell responses to native peptide antigens implicated in the pathogenesis MS and EAE. However, their sensitivity to proteolytic enzymes as well as some immune - mediated side effects impose some difficulties in the use of these agents as MS therapeutics. A number of cyclic myelin peptide analogues seem to be potential candidates in maintaining the biological function of the original peptide and effective in EAE modulation. The potential mmunomodulating and neuroprotective properties of these agents, needs further investigation. Data from studies on EAE models, may introduce clinical trials designed to elucidate the impact of APL in

MS disease activity. These clinical trials should include clinical, neuroimaging and immunological outcomes.

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Monoclonal Antibody Natalizumab for the Treatment of Multiple Sclerosis

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Key words: Multiple sclerosis, treatment, monoclonal antibody, Natalizumab

Activated lymphocytes chemotactically move to the site of inflammation through the vascular system. The process of their extravasation is complicate and includes among others their adhesion to the blood vessel wall through adhesion molecules receptors, VCAM-1 and MadCAM-1. These molecules bind with transmembranic Alpha-4 integrins. Natalizumab is a monoclonal antibody against Alpha-4 integrins, arresting adhesion of lymphocytes on the endothelium, their extravasation and consequently the progress of the inflammatory process, characterizing Multiple Sclerosis.

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Immunomodulation in EAE through Regulation of T Helper Cell Subsets

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Key words: Experimental autoimmune encephalomyelitis, T helper cel subsets, regulation

The possibility to modulate the outcome of a disease by manipulations that induce the selective maturation of T helper cell subsets is a recurrent theme in biomedical research. It has been established, by using different experimental approaches, that favoring the maturation of type 2 helper cells inhibits the progression of CNS inflammation in experimental

autoimmune encephalomyelitis (EAE), the most widely used animal model of the disease multiple sclerosis (MS). Recent developments show that the protective effect might result from alterations on the different T cell pools of the organism such as effector, memory or regulatory T cells. An overview of the current approaches for the possible therapeutic utilization of such knowledge will be presented.

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The Contribution of Docking in the Rational Drug-Design

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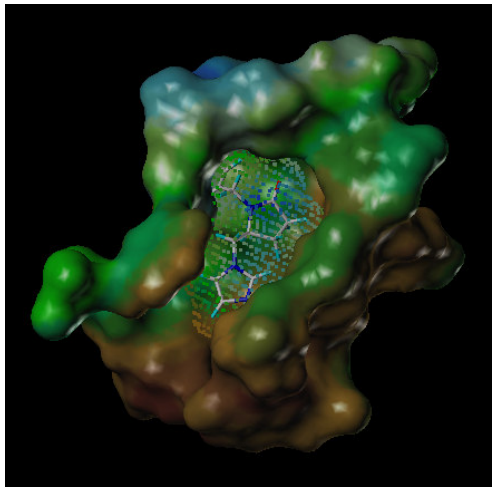
Key words: Rational drug-design, docking



Drugs exert their biological activity by interacting with the active site of the receptor. When the active site belongs to the transmembrane part of the receptor, drug molecules were found to act with a two-step mechanism. First, the molecule is incorporated into the bilayer and then it is laterally diffused in the active site. At the active site the drug interacts with the receptor through specific physicochemical interactions and either blocks the activation of the receptor or triggers a cascade of enzymic reactions responsible for a biological response. The study of the physicochemical interactions between the drug and receptor (docking) can reveal the pharmacophore segments of the drug that

govern its bioactivity and their conformation in space. In addition, it can aid in the design of novel molecules that may exert more productive interactions.

Energy interaction of the antihyrpertensive MM1 in the sheath of human AT1 receptor determined with theoretical algorithms

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Cis-trans Isomerism in Peptides and Proteins: A Structural and Thermodynamic Investigation

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Key words: Peptides, proteins, *cis-trans* isomerism, structural and thermodynamic investigation

Cis-trans isomerism plays a significant role, in conformational-activity relationships in proteins and drugs. At least, one cis-peptide bond was observed, in 6.5% of x-ray protein structures (1). Also, some peptide agonists and antagonists that contain a proline residue were observed to bind on proteins, with a cis-proline conformation. In order to investigate the structural and thermodynamic origin of the cis-trans isomerism and the influence of the environment, the N-methylformamide (NMF) and the bulky N-tert-butylformamide (TBF), simple compounds that contain a peptide bond, were examined by Nuclear Magnetic Resonance (NMR) techniques, as a function of concentration and temperature. Furthermore, some model proline derivatives (AcProOH, AcProNHMe and the ACE inhibitor Captopril) were examined in various solvents and in aqueous solution of different pHs. The thermodynamic parameters: standard free energy (ΔG^0), enthalpy (ΔH^0) and entropy (ΔS^0) of cis-trans isomerism were calculated (2) for the compounds in all solvents. Dimerization was observed in apolar solvents, especially of the cis-form. In some cases, the thermodynamic parameters of the dimerization process were calculated, too. The results clearly show, that at room temperature, as the polarity of the solvent decreases, the cis-form is favored. Also, the existence of a bulky group in the vicinity of the peptide bond increases the percentage of the cis-form. This is in excellent agreement with the high resolution x-ray results in proteins, where cis-peptide bonds were observed mainly in the vicinity of phenylalanine, tyrosine and tryptophane residues (3).

REFERENCES

1. Stewart D.E., Sarkar A., Wampler J.E.: *J. Mol. Biol. 214*: 253-260 (1990)
2. Troganis A., Gerothanassis I.P., Athanassiou Z., Mavromoustakos T., Hawkes G., Sacarellos C.: *Biopolymers 53*: 72-83 (2000)
3. Fisher G.: *Chem. Soc. Rev. 29*: 119-127 (2000)

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Molecular Modeling and NMR Conformational Analysis Apllied in the Study of Zn(II) Binding Sites of Biomolecules

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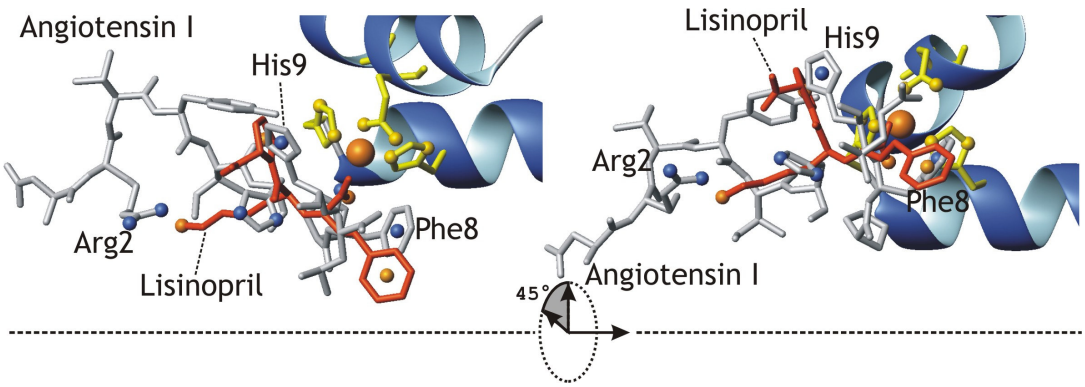
Key words: Biomolecules, Zn(II) binding sites, molecular modelling, NMR conformational analysis



The Angiotensin-I Converting Enzyme (ACE) is a *gluzincin* Zinc Metallopeptidase and plays a pivotal role in blood pressure regulation. ACE catalyzes the hydrolysis of the Angiotensin-I (AI) carboxy-terminal dipeptide His-Leu, thus transforming AI to the vassopressor octapeptide Angiotensin-II (AII). ACE is encountered in two distinct forms in humans, the *somatic* and the *testis* form, bearing 2 and 1 Zn(II)-containing catalytic sites, respectively (1,2). Only recently, the X-ray structure of *testis* ACE has been determined (3).

The reconstitution of the polypeptide skeleton bearing the amino acid sequence of the two catalytic sites of ACE, has been performed using synthetic peptides and Zn(II) salt. The solution structures of these constructs have been studied through Molecular Modeling and high-resolution multinuclear NMR spectroscopy.

The NMR models of two ACE catalytic sites may set the basis in order to study their interaction with physiological substrate, such as Angiotensin I and Bradykinin, as well as with various inhibitors (5).

REFERENCES

1. Ferreira S.H., Bartelt D.C., Greene L.J.: *Biochemistry 9*, 2583-2593 (1970)

2. Ondetti M.A., Williams N.J., Sabo E.F., Pluscec J., Weaver E.R., Kocy O.: *Biochemistry 10*: 4033-4039 (1971)

3. Natesh R., Schwager S.L.U., Sturrock E.D., Acharya K.R.: *Nature 421*: 551-554 (2003)

4. Spyroulias G.A., Galanis A.S., Pairas G., Manessi-Zoupa E., Cordopatis P.: *Current Topics in Medicinal Chemistry 4*: 403-429 (2004)

5. Galanis A.S., Spyroulias G.A., Pairas G., Manessi-Zoupa E., Cordopatis P. : *Biopolymers 76* : 512-26 (2004)

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Computational Analysis of the Conformational Features Induced in Peptide Analogues Containing the (S,S)-CXC- Motif

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Key words: Peptide analogues, (S,S)-CXC- motif, computational analysis

Cyclization via a disulfide bond is a widely used strategy to design constrained peptide analogues. The CXXC motif, which is the smallest and highly conserved unit in biological systems, has been extensively studied to estimate its propensity to form β-turns. Recently we have reported on the synthesis, activity and conformational preferences of highly constraint RGD analogues containing the (S,S)-CDC- motif (1). The main goal of this study is to explore the influence of the (S,S)-CXC- motif to the relative orientation of the X amino acid and the X-2 or X+2 residue side chains. The structure of the peptides has been investigated by molecular dynamics methods. Our findings indicate that despite the nature of the X residue, there is a preference for an almost *cis* coplanar orientation of the X and either one of the adjacent to Cysteine residue side chains. The 11-membered cyclic structure does not favor any β-turn conformation while the backbone dihedral angles within the cycle are very constraint. The χ_3 angle of the first Cysteine residue is distributed around $+80^{o}$ or -100^{o}. It is concluded that the (S,S)-CXC- motif can be incorporated in peptide analogues in which the *cis* coplanar orientation of the corresponding amino acid side chains is desirable. A cluster analysis of the cyclic structures presented here, as well as from other linear RGD containing peptides (2,3), studied by Molecular Dynamics and NMR, further reveals the stabilization effect of the (S,S)-CXC- motif.

REFERENCES

1. Stavrakoudis A., Bizos G., Eleftheriadis D., Kouki A., Panou-Pomonis E., Sakarellos-Daitsiotis M., Sakarellos C., Tsoukatos D. and Tsikaris V.: *Biopolymers 56*: 20-26 (2001)

2. Kostidis S., Stavrakoudis A., Biris N., Tsoukatos D., Sakarellos C., Tsikaris V.: *J. Peptide Sci. 10 :* 494-509 (2004)

3. Biris N., Stavrakoudis A., Politou A.S., Mikros E., Sakarellos-Daitsiotis M., Sakarellos C. and Tsikaris V.: *Biopolymers 69*: 72-86 (2003)

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Molecular Mechanisms Implicated in the Development of Atherosclerosis: The Role of Extracellular Matrix Proteoglycans

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Key words: Atherosclerosis, development, extracellular matrix, proteoglycans, molecular mechanisms

The extracellular matrix (ECM) proteoglycans are molecules that accumulate in atherosclerotic lesions. Their unique molecular features create highly interactive molecules that bind growth factors, enzymes, lipoproteins and a variety of other ECM components to influence fundamental biological events implicated in vascular disease. Proteoglycan synthesized mainly by arterial smooth muscle cells (ASMC) and their synthesis is regulated by specific growth factors and cytokines. The expression of proteoglycans especially that of versican, is markedly upregulated after vascular injury and versican is a prominent component in restenotic lesions.

The interaction of hyaluronan with versican creates an expanded viscoelastic pericellular matrix that is required for ASMC proliferation and migration. Proteoglycans are also accumulated in advanced atherosclerotic lesions, at the borders of lipid-filled necrotic cores as well as at the plaque-thrombus interface, suggesting roles in lipid accumulation, inflammation and thrombosis. Furthermore, the expression of specific versican isoforms influences the assembly of ECM and regulates elastic fiber fibrillogenesis, which is of fundamental importance in ECM remodeling during vascular disease and the development of aneurysms.

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The Molecular Basis of Hypertension by the Use of Biophysical Methods

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Key words: Hypertension, Molecular Basis, Biophysical Methods

In this lecture an atomic level approach of hypertension will be presented. Particular emphasis will be given to:

● The modelisation of the 3D structure of the ACE_N domain of *s*ACE, based on the X-ray structure of testis ACE (*t*ACE).

● Sequence and structural comparison between ACE_N and ACE_C and of other proteins of the gluzincin family highlights key residues that could be responsible for the peptide hydrolysis mechanism.

● Structural models of the interactions of nine ACE inhibitors (lisinopril, captoril, enalaprilat, ramiprilat, quinaprilat, peridoprilat, fosinoprilat, keto-ACE and RXP 407) both to ACE_C and ACE_N catalytic sites were generated by automated computational docking. Pharmacophore refinement, at the atomic level was achieved, which might provide an improved basis for structure-based rational design of second-generation, domain-selective inhibitors.

● Structural studies of the bioactive hormone Angiotensin II (AII) and its inactive precursor AI and implication for the receptor bound conformation trough studies of the monoclonal antibody Fab131 and the homology modeled structure of the GPCRs AT_1.

REFERENCES:

1. Tzakos A.G., Galanis A.S., Spyroulias G.A., Cordopatis P., Manessi-Zoupa E., Gerothanassis I.: *Protein Eng. 16*: 993-1003 (2003)
2. Tzakos A.G., Gerothanassis I.P., Troganis A.N.: *Curr. Top. Med. Chem. 4*: 431-444 (2004)
3. Tzakos A.G., Bonvin A.M., Troganis A., Cordopatis P., Amzel M.L., Gerothanassis I.: *Eur. J. Biochem. 270*,:849-860 (2003)
4. Tzakos A., Gerothanassis I.: *Chem Biochem.*: 2005 *in press*

REVIEW OF CLINICAL PHARMACOLOGY AND
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Initial Experience Using Bone Marrow Derived Stem Cells for Cardiac Reparation Following Large Myocardial Infarction

D.V Cokkinos, A. Manginas, G. Karatasakis, M. Koutelou, A. Theodorakos, N.Kollaros, I. Peristeri, E. Gousetis, M. Theodosaki, S. Grafakos and E. Leontiadis

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Key words: Bone marrow derived stem cells, large myocardial infarction, cardiac reparation, initial experience

Recent reports using intramyocardial (surgical or percutaneous) or intracoronary administration have focused on bone marrow derived stem cells (BMSC) for myocardial regeneration. The latter method of deliver employs an angioplasty balloon catheter, through which BMSC can be slowly infused into the appropriate vascular territory. We describe our preliminary experience using this treatment in patients with old non-viable anterior myocardial infarction and congestive heart failure.

We have treated 12 patients, 10 males, age 50±9, who had experienced an old large anterior myocardial infarction 49±38 months before BMSC treatment. The absence of viability was documented by Tl-201 reinjection scintigraphy and low-dose dobutamine echocardiogram. BMSC were obtained with bone marrow biopsy from the iliac crest during same day procedure, and subsequently processed to isolate a subpopulation (CD +133 cells), known of its capability to differentiate to endothelial cells and probably to cardiomyocytes. A significant number of CD +133 BMSC ($21\pm17x10^6$) were delivered into the coronary artery via a angioplasty balloon catheter. In a group of patients BMSC were labeled in order to document adherence to the myocardium. All patients underwent clinical follow-up, echocardiogram and Tl-201 studies 4.5±2.1 months after treatment.

There were no complications during the procedure or during follow-up. There was no improvement in the global left ventricular ejection fraction, but a significant improvement was noticed in the segmental contractility of the anterior infracted segment. The size of the left ventricle diminished overtime, consistent with favourable remodeling of the heart. These beneficial changes have been associated to the improvement of perfusion in the anterior wall, as shown from the repeat Tl-201 scintigrams.

Infusion of selected BMSC in the coronary artery supplying a large old myocardial infarction results in improvement of local perfusion, segmental myocardial contractility and favourable cavity remodeling. Larger studies are necessary to confirm the safety of this new method of cardiac reparation and the most efficient way to ensure full functional behavior of the implanted cells.

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From Angiotensin to Sartans a New Generation of Anti-Hypertensives

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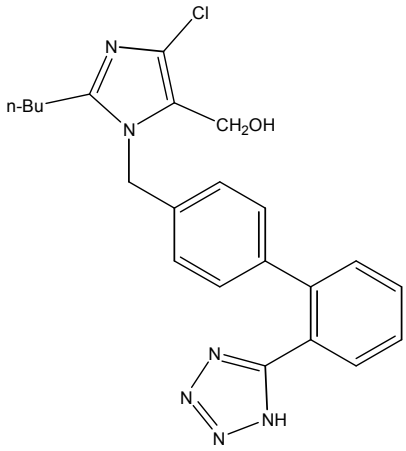
Key words: Antihypertensives, angiotensin, losartan



LOSARTAN

Losartan was the first drug of the Sartan series to be marketed. Using molecular models of Angiotensin II and mimetic technology developed early in 1990's researchers from Patras and Calgary were able to design and synthesize Losartan analogues, which were found to be strong inhibitors. One of them was equally potent to Losartan with longer period of action. However analogue, named Vivartan, was not further developed as synthesis was not cost effective. Latest efforts with structural modifications have reduced steps to achieve a cost effective synthesis of related compounds with strong potency. This presentation will describe methods to design potent ANG II receptor antagonists.

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Selective Phosphinic Inhibitors of Angiotensin Converting Enzyme (ACE)

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Key words: Angiotensin converting enzyme, selective phosphinic inhibitors

Somatic angiotensin – converting enzyme (ACE) contains two homologous domains, each bearing a functional active site with different substrate specificity and different activation requirements (1).

The *in vivo* contribution of each active site to the release of angiotensin II (Ang II) and the inactivation of bradykinin (BK) - two peptide hormones that play a key role in the regulation of blood pressure - is still unknown.

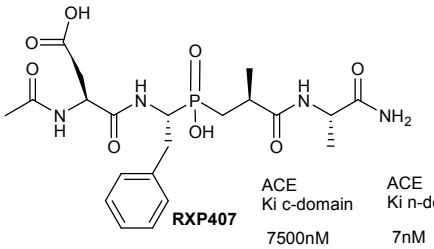
To gain insights into the functional roles of these two active sites, two phosphinic inhibitors RXP407 (2) and RXPA380 (3), were designed and synthesized. These two phosphinic compounds are able to selectively inhibit only one active site of ACE. We will present here: a) the synthetic strategy of the two selective phoshinic inhibitors using combinatorial chemistry and b) the *in vitro* and *in vivo* results.

REFERENCES

1. Corvol P., et al.: *Proc. Natl. Acad. Sci. USA 85*: 9386 (1988)

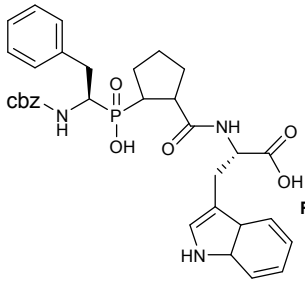
2. Dive V., Yiotakis A., et al.: *Proc. Natl. Acad. Sci. USA 96*: 4330 (1999)

3. Georgiadis D., Yiotakis A., Dive V., et al.: *Cir. Res. 93*: 148 (2003)



RXP407

ACE Ki c-domain	ACE Ki n-domain
7500nM	7nM



RXPA380

ACE Ki c-domain	ACE Ki n-domain
3nM	10000nM

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The Role of Polymorphism of A_2-Adrenergic Receptors in Drug Design

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Key words: A_2-Adrenergic receptors, polymorphism, drug design

Until recently pharmacological intervention at the level of GPCR and α_2-adrenergic receptor was based on three premises:
(a) The pharmacological action was directed to the ligand binding site of the receptor.
(b) The receptor molecule was considered invariant across the population.
(c) Each physiological function was considered to be controlled by a distinct receptor subtype.

All these tenets are under revision. The characterization of signal transduction pathways initiated upon activation of GPCRs allows today the targeting of intracellular effectors for more selective and efficient action. Meanwhile, most GPCRs are polymorphic with the variant forms displaying different biochemical properties.

Alpha$_2$-adrenergic receptors (α_2-ARs) are a representative system in this context. All three known α_2-ARs subtypes (α_{2A}, α_{2B}, α_{2C}) are polymorphic. Genetic variation occurs in the functional third cytoplasmatic loop, thus entailing disruption of the biochemical phenotype of the receptor. Data will be presented showing how genetic differences yield biochemical differences in receptor function correlating with clinical phenotypes.

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Phospholamban's Role in Idiopathic Dilated Cardiomyopathy

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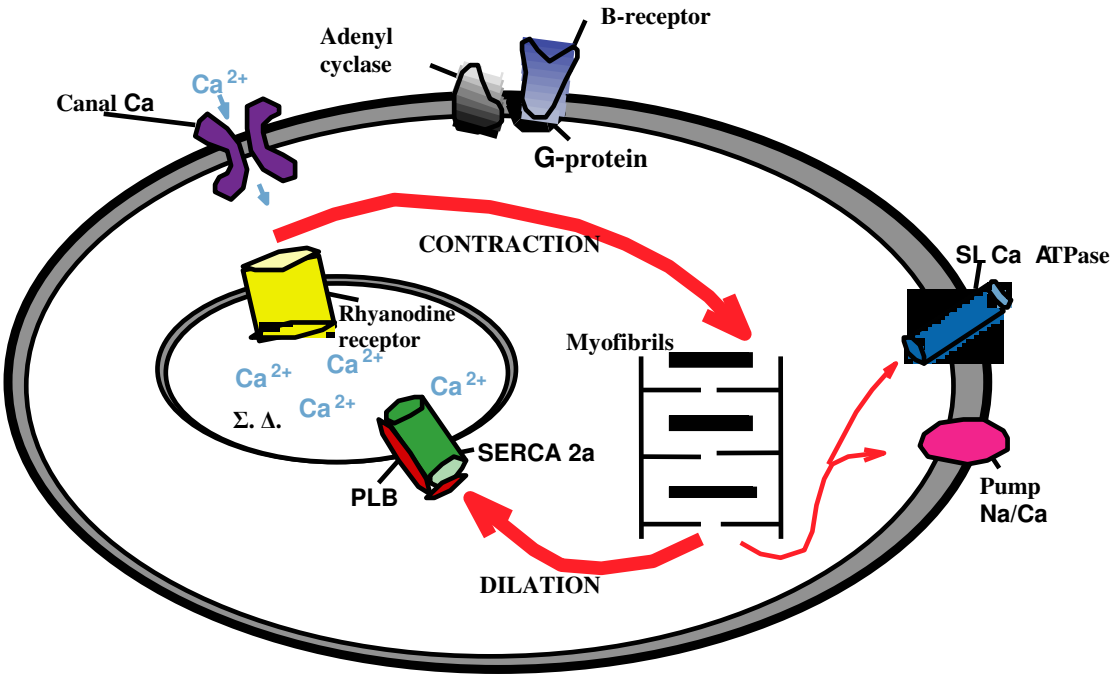
Key words: Idiopathic dilated cardiomyopathy, phospholamban

Idiopathic dilated cardiomyopathy is characterized by dilatation and impaired contraction of the left ventricle or both ventricles as a result of intrinsic cardiomyocyte dysfunction. Altered cardiomyocyte Ca^{2+} cycling is widely recognized as contributing to impaired con-

tractile performance in human and experimental heart failure, including idiopathic dilated cardiomyopathy (1). Coordinated regulation of cytosolic Ca^{2+} by the sarcoplasmic reticulum (SR) of cardiomyocytes is required during each cycle of cardiac contraction and relaxation. During contraction the SR serves as a reservoir from which Ca^{2+} is released into the cytosol via the ryanodine receptor. Sequestration of Ca^{2+} from the cytosol into the SR lumen and, thus, relaxation of the heart is mediated by a sarcoplasmic reticulum Ca-ATPase pump (SERCA2a, Figure). The activity of SERCA2a is reversibly regulated by phospholamban (PLN), a 52-amino-acid phosphoprotein. The role of PLN in the regulation of cardiomyocyte contractility has been elucidated through the development of genetically engineered mouse models in which, PTL ablation resulted in significant increases in cardiac contractile parameters, while PLN overexpression was associated with depressed systolic function (2). Therefore the PTL gene was screened in dilated cardiomyopathy patients and two different mutations were identified recently. The first mutation entailed a conversion of arginine to cysteine (PLN-R9C) in PLN and it was linked to the dominant inheritance of dilated cardiomyopathy in a large American family (3) and the second was a T116G point mutation which resulted in conversion of leucine 39 to a premature stop codon (L39stop) in PLN, that was identified in two Greek families with hereditary dilated cardiomyopathy (4). These data emphasize the role of PTL in cardiomyocyte dysfunction and dilated cardiomyopathy development.



REFERENCES

1. Hasenfuss G.: Alterations of calcium-regulatory proteins in heart failure. *Cardiovasc. Res. 37*: 279-289 (1998)

2. Luo W., Grupp I.L., Harrer J., Ponniah S., Grupp G., Duffy J.J., Doetschman T., Kranias E.G.: Targeted ablation of the phospholamban gene is associated with markedly enhanced myocardial contractility and

loss of beta-agonist stimulation. *Circ. Res. 75*: 401-409 (1994)
3. Schmitt J.P., Kamisago M., Asahi M., Li G.H., Ahmad F., Mende U., Kranias E.G., MacLennan D.H., Seidman J.G., Seidman C.E.: Dilated cardiomyopathy and heart failure caused by a mutation in phospholamban. *Science 299(5611)*: 1410-1413 (2003)
4. Haghighi K., Kolokathis F., Pater L., Lynch R.A., Asahi M., Gramolini A.O., Fan G.C., Tsiapras D., Hahn H.S., Adamopoulos S., Liggett S.B., Dorn G.W., MacLennan D.H., Kremastinos D.T., Kranias E.G.: Human phospholamban null results in lethal dilated cardiomyopathy revealing a critical difference between mouse and human. *J. Clin. Invest. 111*: 869-876 (2003)

REVIEW OF CLINICAL PHARMACOLOGY AND PHARMACOKINETICS, INTERNATIONAL EDITION 20: 402-403 (2006)
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Angiotensin II Receptor Blockade in Cardiovascular Disease

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Key words: Cardiovascular disease, angiotensin II, receptor blockade

Angiotensin II (Ang II) has long been known as a potent vasoconstrictor and stimulator of adrenal steroid synthesis. The coronary, renal and cerebral vasculatures are far more sensitive to the pressor effect of Ang II than vasculature of musculocutaneous tissues. Experimental and clinical work conducted many years ago established the fact that Ang II excess plays a causal role in myocardial and renal damage and is a major contributor to functional deterioration in chronic heart failure. Ang II receptor blockade or angiotensin-converting enzyme (ACE) inhibition were found to increase regional blood flows to vital organs and exert cardioprotective and renoprotective effects. Early studies employed polypeptide analogs/antagonists to Ang II, administered parenterally. Subsequent, far more extensive research work, ranging from basic investigation to multicenter clinical trials, was conducted mostly with ACE inhibitors, whose effects are partly mediated via withdrawal of Ang II and partly via potentiation of bradykinin (which also accounts for some of the adverse reactions to ACE inhibition). More recently Ang II has been recognized to have trophic and mitogenic actions in selected tissues, thus causing hypertrophy and/or proliferation of cardiomyocytes and vascular smooth muscle cells. Chronic ACE inhibition, even in non-hypotensive doses, was shown to reverse left ventricular hypertrophy and vascular wall thickening in hypertensive subjects.

The recent advent of oral Ang II receptor blockers (ABR's) has given new impetus to this field, because comparative experiments using an ACE inhibitor and a selective AT_1 receptor antagonist suggest that the systemic hemodynamic effects of ACE inhibition are mostly due to suppression of Ang II. The favorable hemodynamic properties of ACE inhibition have already established this class of drugs as a treatment of choice in hypertension and congestive heart failure. All ARB's tested so far have the same clinical efficacy in the treatment of these conditions, without the ticklish dry cough which is the most common cause of intolerance to ACE inhibition.

Comparative studies of ARB's against an ACE inhibitor revealed similar decreases of

systolic and diastolic blood pressure and the same response rates on monotherapy, with better tolerability of ARB in all age groups. It has now been shown by several clinical trials that ARB's have long-term cardioprotective and renoprotective effects similar to those of ACE inhibitors in patients with hypertension, ischemic heart disease or diabetes mellitus. Both classes of drugs have also been found to diminish the incidence of new onset type 2 diabetes in patients with hypertension or heart failure.

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Misconceptions in Structural Peptide Chemistry: An Analysis from a Basic Organic Chemistry Perspective

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Key words: Structural peptide chemistry, analysis

The concept of conformation has played a central role in various applications of Organic Chemistry. In this presentation, various misconceptions in conformational structural peptide chemistry are analyzed. More specifically the hypothesis and the conclusions of previous ^{17}O NMR studies on the detection of: (a) discrete conformational states in peptides and (b) of both oxygens of the carboxylic group of Boc-[^{17}O]Tyr(2,6-diClBzl)-OH in DMSO-d_6 solution are reconsidered based on elementary concepts of undergraduate organic chemistry. Furthermore, it is demonstrated that the ^{17}O shielding time scale is not advantageous compared to that of ^{1}H NMR and, thus, it is not suitable for the detection of discrete hydrogen bonded conformational studies on peptides. ^{17}O NMR spectroscopy is prone to interpretation errors due to formation of ^{17}O labeled impurities during the synthetic procedures. The appearance of two discrete resonances at 340 and 175 ppm of this protected amino acid is not now attributed: (a) to the reduction of the intramolecular conformational exchange rate, due to the effect of intramolecular hydrogen bonding of the hydroxyl part of the carboxyl with the carbonyl oxygen of the Boc-group, and (b) to the effect of solvent viscosity, suggested in the mentioned study. The cause of this phenomenon is attributed to a strong hydrogen bonding of the polar proton acceptor solvent DMSO with the carbonyl group, which effectively reduces the proton exchange rate, thus becoming slow on the ^{17}O NMR time scale.

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Polyamines Bind in Close Proximity to RRNA Residues Implicated in the Interaction of Ribosomes with Antibiotics

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Key words: Polyamines, spermine, RRNA residues, ribosomes-antibiotics Interaction

Spermine, one of the naturally occurring polyamines, increases the chloramphenicol and blasticidine S inhibitory effect on protein synthesis by facilitating the drug accommodation to ribosomes (1,2). In contrast, spermine reduces the potency of spiramycin, a macrolide antibiotic (2). To unveil the molecular basis of this polyamine effect, the locations of spermine bound to rRNA were characterized by a photoaffinity labeling technique in combination with RNase H digestion and primer extension analysis. Cross-linking sites of ABA-spermine, a photoreactive analogue of spermine (Fig. 1), were identified in helices H18, H27 and H44 of 16S rRNA, all implicated in the interaction of ribosomes with several antibiotics, such as tetracycline and aminoglycosides. On the other hand, cross-linking was found in helices H35, H42-H44, H89, H90, H95 and the central loop of domain V of 23S rRNA, also involved in the binding of antibiotics which inhibit important ribosomal functions.[3] Ribosomes labeled by ABA-spermine exhibited a behavior towards antibiotics similar to that of native ribosomes interacting with spermine free in solution. Our results suggest that polyamines bound at the vicinity of antibiotic binding pockets modulate diversely the interaction of these drugs with ribosomes.

REFERENCES

1. Xaplanteri M.A., Andreou A., Dinos G.P. and Kalpaxis D.L.; *Nucleic Acids Res. 31*: 5074-5083 (2003)
2. Petropoulos D.A., Xaplanteri M.A., Dinos G.P., Wilson D.N. and Kalpaxis D.L.: *J. Biol. Chem. 279*: 26518-26525 (2004)
3. Spahn C.M.T. and Prescott C.D.: *J. Mol. Med. 74*: 423-439 (1996)

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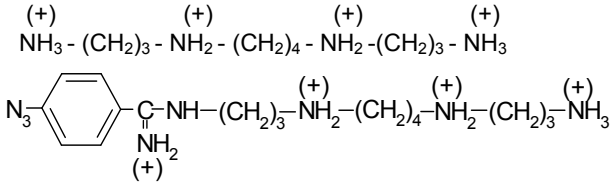

Figure1. Chemical structures of spermine and N^1-azidobenzamidino (ABA)-spermine

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Synthesis of Cyclodextrin–Crown Ether Biomimetic Systems

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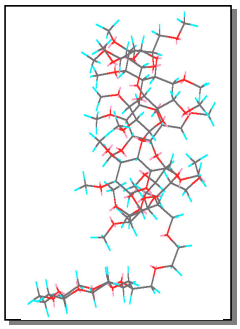
Key words: β-Cyclodextrin, crown ether, biomimetic systems, synthesis

Cyclodextrins (CD) are toroidal shaped molecules that show excellent complexation properties as a result of their unique structure. Thus, cyclodextrins have been used extensively in the construction of supramolecular systems and many examples where cyclodextrin derivatives have served as biomimetic systems in catalysis and/or transport have been already described. Crown ethers, also, represent an important category of supramolecular compounds because of their ability to complex cations.

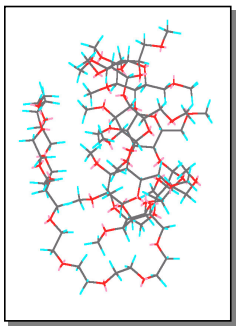
The aim of the present study is to synthesize supramolecular multireceptors and study their ability to complex charged organic molecules such as benzoic and picric acid salts and amino acids. Particularly, molecules that combine permethylated β-cyclodextrin and 18-crown-6 will be presented. The main difference between these molecules is the length of the spacer that connects the cyclodextrin with the crown ether moiety. A short spacer (compound **A** in Figure) does not permit a parallel accommodation of the crown ether with the large rim of the cyclodextrin while a long spacer (compound **B** in Figure) permits such an accommodation. This variation is expected to significantly differentiate the complexing properties of the two compounds.



Ένωση Α

Η επαφή του αιθέρα στέμματος με το μεγάλο άνοιγμα της κυκλοδεξτρίνης δεν είναι δυνατή.

Compound A
The contact of crown ether with the big opening of β-cyclodextrin is not feasible



Ένωση Β

Η επαφή του αιθέρα στέμματος με το μεγάλο άνοιγμα της κυκλοδεξτρίνης είναι δυνατή.

Compound B
The contact of crown ether with the big opening of β-cyclodextrin is feasible

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Thrombin-Angiotensin Mimetics: Design of Double Action Mimetics (Talps: Thrombin Ang II like Peptides)

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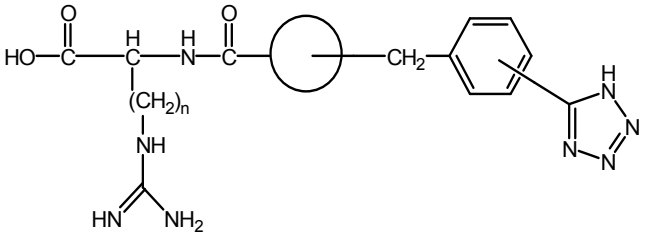
Key words: Thrombin-angiotensin mimetics, double action, design

The results of human thrombin amino acid sequence revealed that the segment 84-135 of thrombin contains two Ang II-like sites. The identified 84-97 and 114-126 sequences are called the *T*hrombin *A*ng II-*L*ike *P*eptides, *TALP*s (TALP I and TALP II, respectively). In this study, we have synthesized a number of non-peptide mimetics, which are designed to carry the pharmacophoric features of the active pentapeptide SFLLR (Phe and Arg) and the crucial pharmacophoric groups of octapeptide Ang II (Phe, His and Arg). The nipecotic acid, the isonipecotic acid (piperidine-4-carboxylic acid) and the cyclohexane-1,4-dicarboxylic acid were chosen as templates upon which the pharmacophoric groups are mounted. Also, all the synthesized compounds will be tested in the rat aorta relaxation assay, in platelet aggregation studies and on the CAM system.

REFERENCES

1. Covic L., Gresser A.L., Kuliopulos A., et al.: *Biochemistry 39*: 5458 (2000)
2. Mavromoustakos T., et al.: *Current Topics in Medicinal Chemistry 4*: 385-401 (2004)
3. Marathias K.P., et al.: *Current Topics in Medicinal Chemistry 4:* 483-486 (2004)
4. Alexopoulos K., Matsoukas J., et al.: *J. Med. Chem. 47*: 3338-3352 (2004)
5. Bone R, et al.: *J. Med. Chem. 41*: 2068 (1998)



Γενική μορφή υβριδικού μορίου με πιθανή συνδυασμένη δράση

General form of hybrid molecule with likely combined action

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Interlaboratory Validation of the In-House Developed Method for the Analysis of the Spasmolytic Hyoscine-N-Butylbromide

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Key words: Spasmolytic Hyoscine-N-butylbromide, analysis, in-house method, validation

According to the legislative frame of the European Union, laboratories must be accredited in order to ensure the quality of analytical results (1). Analytical chemists are allowed to use either internally (in–house) developed methods or significantly modify standard methods (2). In both cases method validation is the process of proving that an analytical method is acceptable for its intended purpose (3). During the development of a new analytical method, the analysts determine the important performance characteristics such as specificity, linearity, precision, measurement range, detection and quantitation limit and robustness (4). Validation must include studies on these characteristics in order to find out whether the method is appropriate or not. Following this framework, validation studies were performed for the determination method of hyoscine–N-boutylbromide in pharmaceutical products employing high performance liquid chromatography. The analysis was conducted using a Supercosil C18 column at 254 nm. The performance characteristics studies demonstrated that the method is scientifically appropriate.

REFERENCES

1. Council Directive 96/23/EK of 12 August 2002 on the approximation of the laws of the Member States relating to units of measurement Official journal L 228, 17/08/2002 p.8.
2. Wood R.: How to validate analytical methods. *Trends Anal. Chem.18*: 624 (1999)
3. Green M.J.: A practical guide to analytical method validation. *Anal. Chem. 68*: 305A (1996)
4. van Zoonen P., et al.: Some practical examples of method validation in the analytical laboratory. *Trends Anal. Chem. 18*: 584 (1999)

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Preparation and Applıcation on Synthesis Hydrophilic Resins

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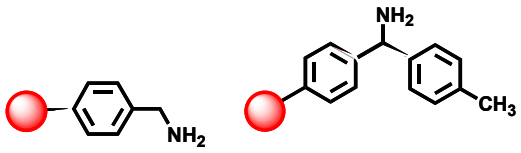
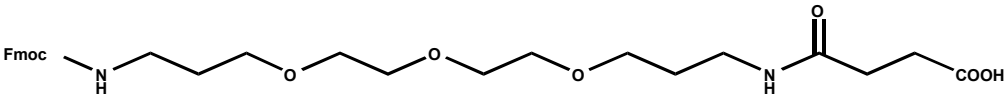
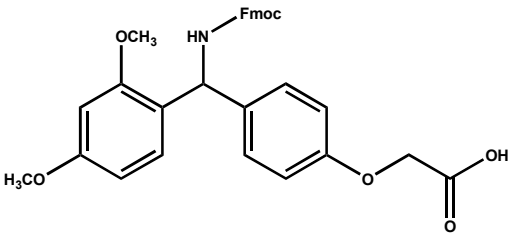
Key words: Hydrophilic resins, preparation, application



AM-PS **MBHA-PS**



Fmoc-PEG-OH



Rink Amide Linker

Solid phase synthesis still possesses a pivotal place in Combinatorial Chemistry. Basic principle of solid phase synthesis is immobilization: a substrate is tightly attached on the resin in the form of a linker. Sometimes, other molecules (e.g. polyethylenoglycol known as PEG) interfere between the resin and the linker, in order to increase the swelling properties of the polymer in polar solvents. The desired chemical reactions take place in a different location of the linker. The selection of the resin we are going to use each time depends on the chemistry demanded for the synthesis of the desired product.

The purpose of this research is the preparation of resins, which demonstrate exceptional swelling properties both in polar and non-polar solvents as well as the applications

of these resins to Combinatorial and Organic chemistry.

Preparation and study of the following resins: (a) A.M –PEG_n-linker (b) A.M-Lys-$(PEG_2)_n$-$(linker)_2$ and (c) MBHA-PEG_n-linker, (linker = Rink Amide Linker, n = 1-10) was carried out concerning the swelling properties in various solvents, kinetic and substitution through synthesis and cleavage of selected peptides (e.g. calcitonin).

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Synthesis of Selected Fragments of the CRF-Related Diuretic Peptide of *Locusta Migratoria*

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Key words: *Locusta Migratoria*, corticotropin-releasing factor (CRF), CRF-related diuretic peptides, synthesis

Locusta Diuretic Hormone (Locmi-DP) is a neuropeptide that has been isolated from the locust Locusta migratoria. It belongs to the CRF (corticotropin-releasing factor)-related diuretic peptides and has the following amino acid sequence.

Locmi-DP stimulates fluid secretion and cyclic AMP production by Malpighian tubules *in vitro* and is released into the haemolymph from corpora cardiaca.

Our aim was to synthesize a series of fragments analogues of the Locmi-DP for structure-activity studies. These fragments are: the C-terminal Locmi-DP_{34-46}, Locmi-DP_{36-46}, Locmi-DP_{38-46} and also the N-terminal [$Hse(Me)^{1,3,13}$]-Locmi-DP_{1-30}, [$Hse(Me)^{13}$]-Locmi-DP_{6-30}, [$Hse(Me)^{13}$]-Locmi-DP_{13-30}.

The synthesis of above analogues was performed in solid phase on the 2-Chlorotrityl chloride Resin using fragments condensation strategy and the Fmoc/Bu^t methodology. The DIC/HOBt method was used for the coupling of amino acids and peptide fragments. The analogues were purified by HPLC and identified by ESI-MS.

REFERENCES

1. Coast G.M., Holman G.M., Nachman R.J.: *J. Isect. Physiol. 36*: 481 (1990)
2. Goldsworthy G.J., Chung J.S., Simmonds S.J., Tatari M., Varouni S., Poulos P.C.: *Peptides 24*: 1607 (2003

Locmi-DP sequence: MGMGPSLSIVNPMDVLRQRLLLEIARRRLRDAEEQIKANKDFLQQI-NH_2

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The Combined Use of CBMN and Fish Methods as a Tool for Cytogenetic Analysis of the Effect of Alkylating Cytostatic Compounds in Human Lymphocytes Treated *in vitro*

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Key words: Human lymphocytes, cytostatic compounds, Cytokinesis-Block Micronucleus (CBMN), fish methods, cytogenetc analysis

The *C*ytokinesis-*B*lock *M*icronucleus (*CBMN*) assay has been widely used in human lymphocyte cultures for the identification of the genetic damage induced by the exposure of cells to various pharmaceutical compounds. By this method Micronucleus frequency is estimated in binucleated cells due to the action of cytochalasin B an inhibitor of actin polymerization. Micronuclei contain acentric chromosome fragments and/or whole chromosomes, they can be recognized as distinct formations that exist in daughter cells separated from the main nucleus and are the result of chromosome breakage and/or chromosome loss (1). The CBMN assay in human lymphocytes in combination with *F*luorescence *I*n *S*itu *H*ybridization (*FISH*) with centromeric probes was proved valuable to identify both chromosome breakage as well as chromosome missegregation induced by various pharmaceutical compounds (2). In the present study we investigated the genetic activity of four cytostatic agents that belong to the category of nitrogen mustards, in human lymphocyte cultures *in vitro*. The studied-molecules are: the pharmaceutical compounds melphalan and chlorambucil, used in the chemotherapy of cancer, the p-mustard of phenylacetic acid, which is the active metabolite of chlorambucil and its steroidal ester ASE, which has been found to exert anticancer activity in experimental tumors (3). We evaluated the ability of the above-referred compounds to induce cytotoxicity and to enhance micronucleus frequency. We also investigated the segregation of chromosomes X and Y with the application of combined CBMN/FISH by using specific centromeric probes. Our results conducted to the conclusion that the studied nitrogen mustards display increased cytotoxicity and induce increased frequencies of micronuclei. In addition they affect the segregation of sex chromosomes, X and Y, increasing chromosome loss and chromosome non-disjunction frequencies. These results are in accordance with previous studies of our group (4).

REFERENCES

1. Fenech M.: *Mutation Research 455*: 81-95 (2000)
2. Bakou K., Stephanou G., Andrianopoulos C., Demopoulos N.A.: *Mutagenesis 17*: 233-239 (2002)
3. Catsoulakos P., Wampler G.L.: *Oncology 39*: 109-112 (1982)
4. Kouloumenta A., Stephanou, G., Demopoulos, NA., Nikolaropoulos S.S. *Anti-Cancer Drugs 16*: 67-75. (2005)

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PHARMACOKINETICS, INTERNATIONAL
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Determinants of the HIV-1 GP120 V3 – CCR5 N-Terminal Interaction at Peptide Level: An NMR Conformational Approach

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Key words: HIV-1 GP120 V3 – CCR5 N-terminal interaction, peptide level, NMR

Recent convincing evidence indicates that the majority of the cells that die due to HIV-1 are not actually infected by the virus. Instead, HIV-1 or its components lead these cells to programmed cell death by altering their physiological function (1) after the activation of apoptotic mechanisms. Ionic interactions between the variable V3 domain of the HIV-1 coat glycoprotein gp120 and the amino terminal of the chemokine receptor CCR5 (2) play a prominent role in this process. Standard multidimensional and multinuclear NMR spectroscopy was applied to probe the structural and physicochemical determinants of three representative peptides from V3 domain of the HIV-1 and a 22-residue peptide, representing the amino terminal of the chemokine receptor CCR5, in their free or interacting state. Titration of CCR5 peptide with V3-peptides was performed in NMR tube, at 286K. 1D 1H NMR spectra and 1H-15N HSQC were recorded after each addition of V3 peptides. Analysis of the NOESY maps, acquired for free and interacting peptides at 278K, suggests that the free CCR5 construct is structured, giving rise to numerous NOEs. Data analysis of HSQC and NOESY spectra verifies the interaction of the V3-CCR5 peptide constructs and suggests a remarkable role for the (i) 7-residue CCR5 Nt domain and particularly for Tyr3 and (ii) for the 7/9-residue V3 N-terminal peptide fragment, which especially in V3 LAI peptide is rich in basic residues (3).

REFERENCES

1. Zafiropoulos A., Baritaki S., Vlata Z., Spandidos D.A., and Krambovitis E.: *BBRC 284*: 875-879 (2001)
2. Baritaki S., Zafiropoulos A., Sioumpara M., Politis M., Spandidos D.A., Krambovitis E.: *BBRC 298*: 574-580 (2002)
3. Rizos A.K., Tsikalas I., Krambovitis E., Sioumpara M., Morikis D., Galanakis P., Spyroulias G.A. *submitted.*

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Synthesis of Tetrazole Analogues of γ- and δ-Amino Acids

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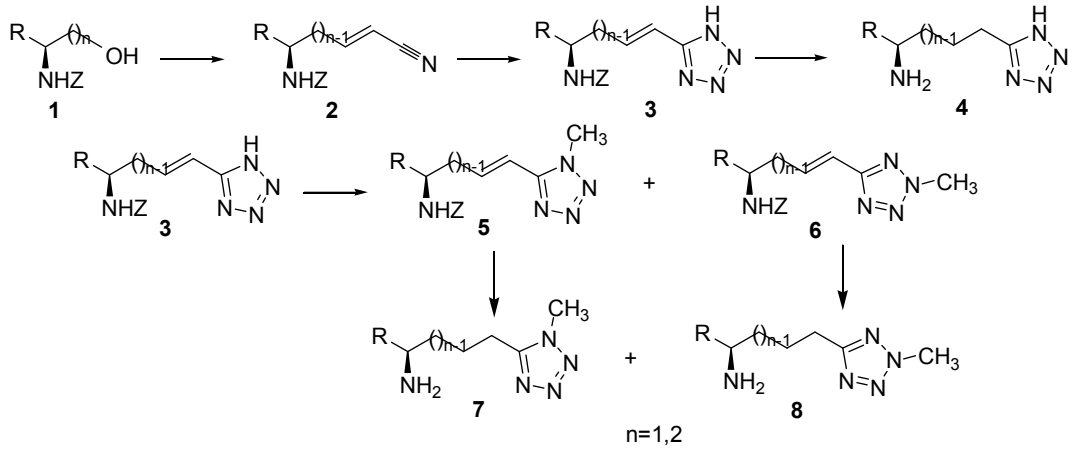
Key words: γ- and δ-Amino acids, tetrazole analogues, synthesis

Non-natural amino acids play an important role in the design and synthesis of pharmacologically relevant molecules and peptide mimetics. The tetrazole group is considered isosteric to the carboxyl group and there are several examples in medicinal chemistry where the replacement of a carboxyl by tetrazole leads to products with improved biological properties. Here, we describe a general method for the synthesis of tetrazole, isosteric analogues of γ- and δ-amino acids and their methylated derivatives, isosteric analogues of aminoacid methylesters. Z-protected amino alcohols 1, easily prepared from α- and β-amino acids, were converted into aldehydes and directly reacted with (triphenylphosphoranylidene)acetonitrile. Treatment of nitriles 2 with NaN_3 and $ZnBr_2$ produced unsaturated tetrazoles 3, which were converted to compounds 4 by catalytic hydrogenation. Treatment of compounds 3 with CH_2N_2 produced the constitutional isomers 5,6 in ratio 1:2, which were converted to compounds 7,8 by catalytic hydrogenation. The constitutional isomers 5,6 and 7,8 can be easily distinguished by ^{1}H-NMR and ^{13}C-NMR.

REFERENCES

1. Herr J.R.: Review, 5-substituted-1*H*-tetrazoles as carboxylic acid isosteres: Medicinal chemistry and synthetic methods. *Bioorg. Med. Chem. 10*: 3379-3393 (2002)



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Pepticom Hellas: Steps Forward Research and Innovation *Multidimensional Applications and Perspectives*

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Key words: Pepticom Hellas, multidimensional applications and perspectives

Nowadays, the use of synthetic carriers as biochemical reagents and immunogens is entering to a new phase. The multimeric nature of these constructs, the unambiguous composition and the ease, reliability, versatility of their production make this type of carriers well suited to various biotechnological and biochemical applications for diagnostic purposes, protein mimetics, antiviral agents, vaccines, drug and gene delivery vehicles. We will be focused on an innovative type of multifunctional helicoid carrier-foldamer, named *SOC_n-I, II,* which was successfully developed in our laboratory. Our concept was to construct an artificial template with structural rigidity and regularity, so as the peptide epitopes/pharmacophore groups could be anchored without any conformational restriction and steric hindrance as demonstrated by conformational studies using ^{1}H-NMR, CD, FT-IR. SOC_n-I,II were used as antigenic substrates in developing immunoassays of high sensitivity, specificity and reproducibility, as well as potent immunogens to generate site-specific antibodies for therapeutic purposes. By exploiting the ability of macromolecules SOC_n-I,II to act as potent antigens, it would be possible to develop diagnostic tests (as for example an immunochromatographic lateral flow test), specific in screening and discrimination among various autoimmune diseases, which enable us to simultaneously detect various antigen and antibody combinations. In the case of massive analyses (as for hospitals, diagnostic centers) our macromolecules SOC_n-I,II could be served in developing a kit based on turbidimetry or nephelometry principles applicable in biochemical analytes.

Taking advantage of the SOC_n properties to generate site specific antibodies (potent immunogens), we have emphasized on the application of SOC_n carriers, covalently bearing a 'built-in' adjuvant moiety, for the preparation of totally synthetic peptide-based vaccine matrix for human use. Thus, according the selected epitope/pharmacophore group to be attached on this matrix, we could prepare multiple synthetic human vaccines for therapeutic potential.

On the other hand, the structural characteristics of SOC_n-I,II carriers, render them a useful tool for the study and comprehension of mechanism of interaction with membranes. To this direction, the SOC_n-I,II carriers could be tested not only as antimicrobial agents, but as tools for gene delivery as well.

Our increasing understanding of peptide/protein folding and assembly, raises possibilities for engineering novel self-assembling supramolecular structures and bio inspired materials. Potential applications of such assemblies, include the preparation of functionalized biomaterials of nano-dimension (nanomaterials), as well as scaffolds to recruit cells

for cell/tissue engineering. Based on the rational design principles, we could synthesize carriers of lipid moieties, in order to form well characterized amphipathic structures and self-assembled molecules.

Furthermore, we mention the exploitation of the amphipathic structure of a new class of molecules in synthesizing HDL models, which interact with lipoproteins, involved in cholisterinemia. We rationally designed a-helical amphipathic peptide models as mimics of HDL amphipathic helices in order to prevent LDL oxidation and thus generate potent anti-atherogenic agents. Indeed, our constructs could be served as atheroprotective candidates in the field of cardiovascular diseases.

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Design and Synthesis of MBP_{83-99} Epitope Conjugated with Mannan for the Immunotherapy of Multiple Sclerosis

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Key words: Multiple sclerosis, immunotherapy, MBP_{83-99} Epitope, synthesis

Muliple Sclerosis (MS) is the most common autoimmune disease of the Central Nervous System (CNS). There is evidence that MS patients have Th1 immune response with secretion of inflammatory cytokines like IL-2, IFN-γ, TNF-α. A new strategy for the treatment of MS is to switch the immune response from Th1 to Th2 with secretion of anti-inflammatory cytokines like IL-4 and mostly IL-10. Experiments have shown that peptide analogues conjugated with the oxidized form of mannan lead to Th1 immune response whereas reduced form of mannan leads to Th2 immune response. The aim of this project is to modify the Th1/Th2 ratio by conjugating the MBP_{83-99} epitope with oxidized and reduced mannan.

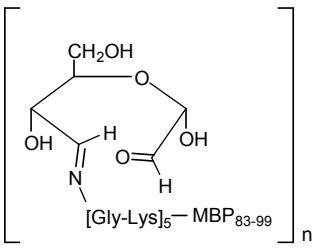
REFERENCES:

1. Apostolopoulos V., Barnes N., Pietersz G.A., McKenzie I.F.: *Vaccine, 18*: 3174 (2000)

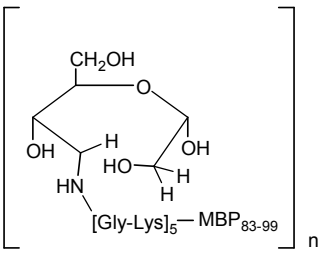
2. Bielekova B., Goodwin B., Richrt N., Cortese I., Kondo T., Afshar G., Gran B., Eaton J., Antel J., Frank J., McFarland H., Martin R.: *Nature Medicine 6*: 1167 (2000)

3. Kappos L., Comi G., Panitch H., Oger J., Antel J., Conlon P., Steinman L.: *Nature Medicine 6*: 1176 (2000)

4. Tselios T., Apostolopoulos V., Daliani I., Deraos S., Grdadolnik S., Mavromoustakos T., Melachrinou M., Thymianou S., Probert L., Mouzaki A., Matsoukas J.: *J. Med. Chem. 45*: 275 (2002)



Oxidized Mannan



Reduced Mannan

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Bridging CD4+ and CD8+ Epitopes for Anti-Cancer Vaccine Design

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Key words: Anti-cancer vaccine, CD4+ and CD8+ epitopes

Anti-cancer antigen responses have been detected in many tumor patients. CD4+ and CD8+ epitopes have also been identified, but their responses are not accompanied by tumor regression. In order to improve the cancer vaccine strategy, we propose the design and preparation of conjugates, which incorporate CD4+ and CD8+ epitopes from the same cancer antigen for testing their potency to elicit anti-tumor responses.A Sequential Oligopeptide Carrier, SOC_4, formed by the repeating tripeptide Lys-Aib-Gly, was synthesized on a Wang resin by the Fmoc strategy. Lysines were introduced as Fmoc-Lys(Alloc)-OH and Fmoc-Lys(Mtt)-OH. This orthogonal protection allowed the synthesis of different epitopes on the Lys-$N^{\varepsilon}H_2$ groups. After the removal of the Mtt group (1.8% TFA in DCM), the CD8+ epitope was synthesized. The synthesis of the CD4+ epitope was performed by the Fmoc strategy after the catalytic removal of Alloc by $Pd(PPh_3)_4$. The obtained conjugates, after cleavage from the resin with TFA, were purified by HPLC and identified by ESI-MS. Biological studies for evaluating T cell stimulation are currently in progress.

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Development of a Capillary Electrophoretic Method for the Determination of Antioxidants and Malondialdehyde in Biologic Samples

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Key words: Antioxidants, malondialdehyde, biologic samples, determination, capillary electrophoretic method

Oxidative stress is involved in the pathophysiology of many ocular disorders, such as age-related macular degeneration, cataract and glaucoma. Tears and aqueous humor nourish and protect the eye and are rich in antioxidants, such as ascorbic acid, uric acid, tyrosine, cysteine and glutathione. The aim of the present work was to develop a novel method for the simultaneous determination of three antioxidant molecules (ascorbic acid, uric acid and tyrosine) and of MDA (lipid peroxidation indicator) both in reflex tears and aqueous humor by high performance capillary electrophoresis. Tears were collected with Schirmer strips and the aqueous humor samples following surgical procedure. After pilot experiments, optimum separation is achieved in a 25 mM borate buffer, pH 10.0, containing 100 mM sodium dodecyl sulfate (SDS) at the temperature of 25 °C and 20 kV (normal polarity). The developed HPCE method has good repeatability, precision and high sensitivity.

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