Dear readers,

The Hellenic (Greek) Society of Pharmacology was founded in 1984 with the aim to promote research in Pharmacology in Greece – in the experimental and clinical setting – and to advance the communication between all medical doctors and scientists who have an active interest in drugs and their mode of action.

The Hellenic Society of Pharmacology holds its Panhellenic Congress once every two years and a Meeting in the intervening years. Either occasion serves as a forum in which, members of the Society and other interested scientists, have the opportunity to exchange information – in the form of lectures, round tables, oral and poster presentations - concerning various aspects of drug development and use, from basic research to the legal setting covering their therapeutic administration.

This is the first time that abstracts from a Congress/Meeting of the Hellenic Society of Pharmacology are published in this journal. We do hope that our readers will find them useful and intellectually stimulating.

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ORAL PRESENTATIONS

O1 EFFECT OF TRANSFORMING GROWTH FACTOR B AND BONE MORPHOGENETIC PROTEIN 2 ON PROTEOGLYCAN EXPRESSION BY HUMAN PRIMARY PULMONARY ARTERIAL SMOOTH MUSCLE CELLS

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The transforming growth factor (TGF)-β family is a large family of multifunctional cytokines playing critical roles in embryogenesis, growth, wound repair, inflammation and vascular homeostasis. Bone morphogenic proteins (BMPs) are the largest group of cytokines within the TGF-β superfamily. BMPs act as instructive signals during embryogenesis and contribute to the maintenance and repair of adult tissues. Both TGF-β and BMP isoforms are pleiotropic mediators of smooth muscle cell proliferation and apoptosis, as well as extracellular matrix (ECM) secretion and deposition. Proteoglycans are essential ECM molecules, which modulate inflammatory responses and influence tissue repair and remodeling. The aim of our study was to investigate the effect of TGF-β and BMP-2 on the expression of proteoglycans by human pulmonary arterial smooth muscle cells (PASMC).

PASMC were incubated for 6, 12 or 24 h in the presence of 0, 0.2, 2 and 10 ng/ml of TGF-β, as well as for 0, 2 and 6 h in the presence of 10 ng/ml of BMP-2. At the end of the incubation period, the RNA was isolated and gene expression of the proteoglycans biglycan, perlecan, decorin, syndecan and versican was analysed by RT-PCR. Human hydroxy-methylbilane synthase, a ubiquitously and equally expressed gene free of pseudogenes, was used as a reference gene in all RT-PCR reactions.

We found that gene expression of biglycan, perlecan, syndecan and versican was significantly stimulated by TGF-β, after 24 h of incubation, in a dose dependent manner, whereas, gene expression of decorin was significantly downregulated. BMP-2 significantly induced gene expression of perlecan, syndecan, versican and decorin but not of biglycan.

Our results show an upregulation of specific proteoglycan gene expression in response to TGF-β and BMP-2, which may play a significant role in vascular remodelling, associated with pulmonary diseases, such as idiopathic pulmonary arterial hypertension (IPAH) and they support the rationale that they may serve as alternative targets for pharmacological intervention to prevent and/or to treat vascular pulmonary diseases.

O2 IDIOPATHIC ARTERIAL PULMONARY HYPERTENSION IS ASSOCIATED WITH DIFFERENTIAL EXPRESSION OF THE PROTEOGLYCAN VERSICAN, DECORIN AND PERLECAN IN THE LUNG

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Idiopathic pulmonary arterial hypertension (IPAH) is a fatal disease characterised by vasoconstriction, proliferation of pulmonary arterial smooth muscle cells (PASMCS) and increased deposition of extracellular matrix (ECM), which contributes to pathological remodelling of pulmonary arterioles by 48% and of perlecan (by 45%) in IPAH lung tissue specimens, as compared to controls, indicating disruption of the rigidity of parenchymal lung tissue and of the basement membrane. In contrast, gene expression of decorin was upregulated by (20%), in IPAH, as compared to controls. Since decorin modulate collagen fibrillogenesis, lung tissue mechanics may be affected through a direct effect of decorin on collagen fibril formation.

Our results indicate a possible involvement of PGs in IPAH pathophysiology with future implications in the prevention and treatment of the disease.

O3 MDEA: IN VITRO STUDY OF THE EFFECT ON HUMAN GENETIC MATERIAL

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MDEA (3,4-Methylenedioxy-N-ethylamphetamine, “Eve”) is a psychoactive drug which acts upon the central nervous system affecting brain function. This way, it causes changes similar to those of MDMA (3,4-methylenedioxymethylamphetamine) in perception, mood, behavior and cognition. MDEA which belongs to the phenethylamine amphetamine chemical classes is also psychedelic and stimulant. It is a white crystalline powder, completely soluble in water. Like MDMA and related methylenedioxyphenethylamines (such as MDA, MBDB and BK – MDMA), MDEA acts as a norepinephrine, dopamine and serotonin releasing agent. Usually MDEA requires larger dose than MDMA to cause the same phenomena and its effects last approximately three to five hours. Nevertheless, the euphoric feelings caused by MDEA are not as intense as those caused by MDMA. Furthermore, the effects of MDEA are less stimulating than those caused by MDMA. In this experimental work we study the effect of MDEA on human DNA by estimating the most sensitive and accurate cytogenetic indices, SCEs (Sister Chromatid Exchanges), PRI (Proliferation Rate Index) and MI (Mitotic Index). SCEs are considered as one of the most sensitive markers of genotoxicity, whereas PRI is used as a reliable index of cytototoxicity, though MI is a measure for the proliferation status of a cell population. We prepared five MDEA concentrations (the middle one is counterpart to the concentration found in the blood of a regular user), which were added to lymphocyte cultures of peripheral blood from young, healthy donors. After 72 hours incubation the cultures were prepared and stained by Fluorescence plus Giemsa method and SCEs, PRI and MI were estimated using an optic microscope. MDEA caused significant cytogenetic damages (increase in SCE frequencies and decrease in PRI values) on the lymphocytes which were proportional to the concentration of drastic substance. The observed differences in some cases were statistically significant. These results lead to the conclusion that the effect of MDEA on genetic material is an impressive field which should be further investigated.

O4 CYTOSTATIC BEHAVIOUR OF CROCIN ON LEUKEMIC CULTURED LYMPHOCYTES

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Introduction: Crocin is isolated from saffron, an important herb rich in carotenoids obtained from the stigmas of Crocus sativus L, commonly consumed in different parts of the world and used as a medical drug to treat numerous diseases. Crocin is the diester formed from the disaccharide gentiobiose and crocetin and it has been shown to have antioxidant, antidepressant and antiangiogenic effects. Our results indicate a possible involvement of PGs in IPAH pathophysiology with future implications in the prevention and treatment of the disease.

In the present work a comparative study of the cytogenetic behaviour of crocin between cultured lymphocytes from leukemic patients as well as from healthy individuals was undertaken in order to test the hypothesis that the Sister Chromatid Exchange (SCE) assay in vitro can be used for the prediction of in vivo tumor response to the potential chemotherapeutic action of crocin. SCEs have been proposed as a very sensitive method for detecting genotoxicity, and lately as one of the methods for
evaluating chemotherapeutic efficiency in vitro and in vivo, while Prolif-
eration Rate Index (PRI) has been established as a valuable indicator of
cytostatic effect.

Methods: Lymphocyte cultures have been prepared by adding: a) 11-12
cytostatic effects. The proliferation rate (PRI) has been established as a valuable indicator of
evaluating chemotherapeutic efficiency in vitro and in vivo, while Prolifer-
ations of crocin solutions.

Results: Findings showed that all tested crocin solutions didn’t cause re-
markable changes to the PRI values neither of the leukemic, nor of healthy
lymphocytes. Contrariwise, after crocin affection a statistically significant
decrease of the SCE frequency of leukemic lymphocytes had been ob-
served, though the SCEs of healthy cells presented slight increase. Both
the reduction and the increase of SCEs were proportional to the concentra-
tions of crocin solutions.

Discussion: In conclusion crocin didn’t cause significant changes to the
proliferation rate of leukemic lymphocytes and therefore it didn’t prove to be cytostatic in the tested concentrations, but mainly it reduced significant-
ly the DNA damages along with being demonstrated as cytoprotective.

O5  IN SILICO VIRTUAL HIGH-THROUGHPUT SCREENING AP-
PROACH IN PROFILING THE DRUG POTENCY OF VARIOUS
SAFFRON BIOACTIVE CONSTITUENTS

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Introduction: In silico virtual high-throughput screening is a new branch of
medicinal chemistry that represents a reliable, timesaving and cost-
effective tool for computationally screening databases, for the discovery
of novel drug leads. This approach has become increasingly popular in
the pharmaceutical research. Saffron is a well-known spice in traditional
medicine, with many reputed therapeutic uses, including its use as a tonic,
nerve sedative, antioxidant, anti-depressant and against dementia. In this
study we explored the drug-potency of some saffron constituents.

In silico computational methods: All compounds have been screened vir-
tually, against a large protein drug target database comprising over 1,000
target-proteins. Receptor-stand molecular docking used, is a computational
tool of structure based drug design, to predict protein-ligand interaction geo-
metries and binding affinities. The produced compound-protein complexes
were ranked by the energy score, including their binding conformations.

Results: For each compound, at least 20 target-proteins were found to be in-
hibited in a specific order of binding capacity. Our docking findings, in
many cases, support the biological data for the saffron’s compounds. The
proteins that were found to interact with crocin, crocetin and safranal, are
acetylcholinesterase, coagulation factor VIIa, IX and X, serine proteinase,
tripsin, neutrophil collagenase, MMP8, thymidine kinase, beta-glucosidase,
thrombin, acoustate hyd Ryderase, NADPH dehydrogenase, phosphoglycer-
ate dehydrogenase family 4 member C, calmodulin, methylmalonylcoenzyme
methyltransferase, methionine synthetase, elongation factor Tu, alpha-amylase,
thymidylate synthetase, RNA tri-
phosphatase, tubulin, casein kinase II, protein kinase ck2, human neutrophil
ghesphatase and dehydrogenase reductase. These proteins are involved in many
diseases as Alzheimer’s disease, coronary atherosclerosis, various cancers,
thrombotic disease, coagulative disorders, atopic asthma, cardiovascular
disease, myeloneuropathy, anemia, folate acid deficiency, homocystinuria,
malaria, Parkinson’s disease, hypoxic-ischemic encephalopathy, cognitive
deficits, herpes virus infection, trichomoniasis, motor neuron disease, acne
vulgaris, endometriosis, Parkinson disease and others.

Conclusion: Our in silico molecular docking calculations provided a mo-

cular basis for understanding the inhibitory effect of the studied com-

pounds on various proteins, implicated in a large number of diseases. These
results may be of value for the development of novel therapeutic agents
based on carotenoid-based inhibitors.

O6  DISTRIBUTION OF THE INOSINE TRIPHOSPHATASE (ITPA)
94C>T AND IVS2+21A>C GENE POLYMORPHISMS IN THE GREK
POPULATION

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Introduction/Aim: Inosine triphosphatase (ITPA) is a cytoplasmic pyro-
phosphatase which recycles IMP back to IMP and thus makes it available
for the salvage purine pathway. The ITPA gene is located on chromosome
20p13 and consists of 8 exons. Two single nucleotide polymorphisms
(SNPs; ITPA94C>A and IVS2 + 21A>C) are associated with reduced en-
zyme activity, with an established phenotype – genotype correlation. The
two SNPs have been associated in the past with thiopurine and methotrex-
ate drug toxicity in patients with inflammatory bowel disease (IBD), and,
more recently, with protection against haemolytic anaemia in hepatitis C–
infected patients treated with peg-IFNα/ribavarin. As those two SNPs are of
obvious pharmacogenetic interest, we sought to examine their distribution
in the Greek population for which such data are not available, as yet.

Materials and methods: DNA was isolated from peripheral leukocytes of
88 apparently healthy individuals of Greek ethnicity. Both SNPs (ITPA
94C>A and IVS2 + 21A>C) were genotyped using a PCR-RFLP method.
Results: There was no statistically significant deviation from the Hardy-
Weinberg equilibrium with respect to either SNP, in the sample examined.
The frequency of the ITPA 94A allele was 0.09, whereas that of the ITPA
IVS2 + 21C allele, 0.125. Only two homozygotes for the minor allele
were detected, one for each polymorphism. Based on our genotyping and
projecting to the Greek population, at least 37.5% of the Greek population
disesplays some extent of genetically determined ITPA deficiency and ap-
proximately half of those individuals could express 30% or less of the aver-
age ITPA activity of homozygotes for the major alleles of the ITPA SNPs.

Conclusions: A significant percentage of the Greek population carries a ge-
netically determined deficiency of the ITPA enzyme, similarly to other Eu-
ropean or American Caucasians. Genotyping IBD or hepatitis-C patients
for the two SNPs primarily associated with this deficiency may be of clini-
cal value in predicting response to thiopurines or ribavarin, respectively.

O7  AN ASSESSMENT OF THE PERCENTAGE OF PATIENTS AT POSSI-
BLE RISK FOR CLOPIDOGREL NON-RESPONSIVENESS, BASED
ON THE PREVALENCE OF CYP2C19*2 AND ABCB1 C3435T GENE
POLYMORPHISMS IN THE GREEK POPULATION

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Introduction/Aim: Clopidogrel is a widely used antiplatelet pro-drug which
undergoes metabolic activation in the liver. A common single nucleotide
polymorphism (SNP) in the gene coding for cytochrome P450 2C19, CYP2C19*2
was repeatedly shown to be associated with clopidogrel non-
responsiveness, due to inadequate production of the active metabolite.

Other well characterized polymorphism, C3435T in the ABCB1 (MDR1)
gene, has also been implicated in poor response to clopidogrel, in at least one
large study. We have genotyped a sample of Greek individuals in an attempt
to gather data pertaining to an assessment of the percentage of Greek patients
that may be at risk for inadequate response to treatment with clopidogrel.

Methods: One hundred and eight unrelated individuals of Greek ethnic ori-
gin, all apparently healthy, were genotyped for the ABCB1 C3435T and the
CYP2C19*2 SNPs, using established RFLP methods.
Results: There was no statistically significant deviation from the Hardy-Weinberg equilibrium, with respect to either polymorphism. The genotype and allele distributions of the ABCB1 C3435T polymorphism was similar to those reported for some other Caucasian populations (ABCBI T allele frequency = 0.58), whereas those of CYP2C19*2 were close to results previously reported for the Greek population (CYP2C19*2 allele frequency = 0.19).

Conclusion: By taking into consideration CYP2C19*2 genotyping only, an estimated 34% of the Greek population (carriers of the CYP2C19*2 allele) may be at risk of inadequate response to clopidogrel. This figure can increase considerably if ABCB1 TT genotypes are taken into account.

O8 INCIDENCE OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE ADH1B, ADH4, ADH1C, OPRM1, DRD2, BDNF AND ALDH2 GENES IN THE GENERAL POPULATION AND CORRELATION TO ALCOHOL AND NICOTINE DEPENDENCE

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Background: Differences in genes may cause different responses to drugs, due to alternate pharmacodynamic and pharmacokinetic drug’s effects. In the present work we examine Single Nucleotide Polymorphisms which could influence the response to alcohol and nicotine, their frequency in the non-addicted population and the combined influence of these SNPs with a total genotype score for each volunteer.

Methods: Using a database with the genotype analysis of 308 people, containing data for 171 SNPs, a bibliographical research was made in order to extrapolate which SNPs are related to nicotine and/or alcohol addiction. The various genotypes for highly related genes from the database was calculated to estimate the rate of occurrence of each SNP in a hellenic, non-addicted population.

Results: The bibliographical research indicated that 7 of the investigated SNPs are related to nicotine and/or ethanol addiction. SNPs of the metabolic enzymes genes ADH1B, ADH4, ADH1C and ALDH2 and the re-ward pathway in CNS associated genes BDNF, OPRM1 and DRD2 were investigated. Volunteers participated in the study are homozygous for the alleles with these SNPs at a rate ranging from 0% to 17% and heterozygous at a rate reaches up to 51%. The total genotype score (TGS) was calculated using an algorithm for ethanol and nicotine and we found that the majority of the volunteers has a TGS below the middle of the range 0-100.

Conclusions: The present study demonstrated that alcohol and nicotine addiction is associated with SNPs of genes involved in the metabolism and the action of these drugs. Furthermore, all of these SNPs, but the SNP of ALDH2 gene, were found in the genome of a hellenic population. Finally, volunteers have a low genetic potential for addiction, because of the low TGS for each substance.

O9 THE ROLE OF THE MULTICANCER MARKER RS6983267 IN CIGARETTE SMOKE EXPOSED PATIENTS WITH PROSTATE CANCER

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Introduction: Common variants on human chromosome 8q24 were found to be associated with prostate cancer risk with different frequency and incidence among the investigated populations. We examined the effect of smoke on this type of cancer and its relationship with the risk variant rs6983267, located at region 3 of chromosome 8q24, in a prostate cancer case-control study conducted in the Greek population in light, intermediate and heavy smokers.

Materials and methods: Samples of total blood from 74 patients with histologically confirmed prostate cancer and 24 healthy individuals were genotyped using real time polymerase chain reaction (PCR). Tumor-node-metastasis (TNM) stage, Gleason score and levels of prostate-specific antigen (PSA) at diagnosis were included in the analysis.

Results: Light (Packyears, PY<10) and heavy (PY>30) smokers are positive associated with prostate cancer, with an additive risk for the carriers of rs6983267 with positive smoking history (ORadj=21.21, C.L=1.79-119.92) to develop the disease.

Discussion: In our study, homozygotes or heterozygotes had 2.84 times greater likelihood for PCa (p=0.002) and the overall population frequency for the G allele was 61.85%. The carriers had almost two times greater odds for having the G allele (p=0.001) with a sensitivity for the disease of 81.40%.

In conclusion, our findings support the established model for PCa, of being a complex disease with genetic and environmental factors contributing to the carcinogenesis through different mechanisms. The SNP, rs6983267, has an independent risk for carriers to develop prostate cancer and in combination with smoke; it confers additive risk for the disease, similarly to others, well established risk factors such as age, family history and ethnicity.

O10 IN VIVO EVALUATION OF CYP1A2 ACTIVITY IN GREEK HEALTHY VOLUNTEERS BY THE RP-HPLC QUANTIFICATION OF CAFFEINE METABOLIC RATIOS IN SALIVA AND URINE SAMPLES

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Introduction: Human CYP1A2 activates metabolically a great number of procarcinogens to active intermediates and is responsible for the metabolism of many clinically used drugs. Caffeine, a drug with the largest consumption among humans, is commonly used as a probe drug for the simultaneous assessment of the phenotypes of various drug-metabolizing enzymes, including CYP1A2. In the present study a RP-HPLC method was developed for the assessment of caffeine (137 MX) and paraxanthine (17 MX) in saliva samples in order to evaluate CYP1A2 in vivo activity in human volunteers.

Methods: Spot saliva samples were analyzed 6 h after 200 mg caffeine consumption, following a 12 h methylxantine-free diet. CYP1A2 activity was evaluated from the metabolic ratio 17X/137X. Metabolites and the internal standard (IS) were extracted with chloroform/isopropanol (85:15, v/v) and separated on a C18 column by an isocratic HPLC system with mobile phase comprised of 0.1% acetic acid-methanol-acetonitrile, 80:20:2 v/v and detected at 273 nm.

Results: The method exhibited adequate separation of caffeine and its major metabolites paraxanthine, theobromine, theophylline as well as the IS paracetamol (resolution factors >2.8), bias (-1.7 - 8.6%) and intraday and interday precision <6.8% (n=6). The recoveries of paraxanthine and caffeine, respectively (Signal to Noise ratio = 3). The limit of quantitation, for both substances, was 0.1 μg/ml with precision and accuracy 15% or better. The developed RP-HPLC method was fully validated and successfully applied for the evaluation of CYP1A2 activity by recruiting 22 Greek healthy volunteers. Median values (range) of metabolic ratios for smokers and non-smokers were 0.85 (0.31-1.39) and 0.37 (0.17-0.66), respectively (p=0.001). Saliva ratios with the highest value and significantly correlated with urine caffeine metabolite ratios assessed in the present population of healthy volunteers (Pearson correlation coefficient 0.894, p=0.001) (for complete validation methodology of urine caffeine metabolite ratios see Begas et al., 2007).

Conclusion: The caffeine test developed in the present study is a non-invasive, well-tolerated, easily accessible method for assessing the in vivo activity of CYP1A2 in population studies of healthy subjects and for monitoring CYP1A2 impairment in patients with liver disease.
### O11 MEDICATIONS AS A SOCIAL GOOD. COST/EFFECT. EXPERIENCE WITH THE USE OF GENERICS IN Aretaieio UNIVERSITY HOSPITAL, ATHENS GREECE

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Medication use except for their social aspect has had also a great impact on financial issues since ancient time. In the 13th century a.C. in an effort to reduce drug overprescription, medical profession was officially separated from that of the pharmacist. With the rise in life expectancy the treatment cost of many progressive degenerative and chronic diseases tends to a tremendous increase as well. New biotechnological methods in drug preparation claim long – term research and high financial investments resulting in very expensive medications. Medication availability and access steps are: production - prescribing – purchase - consume. Under the social demand for unlimited health budgets it is estimated that the medications expenses have an annual rise of 5% in western countries. The growing use of generics could be considered as a means to control the rising health care costs. In developed generics markets (British, German) their consumption overrides the 50% of the total medication sales.

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<th>Medication</th>
<th>Cost restriction</th>
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<td>52% generic</td>
<td>Brand offer lower price 26%</td>
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<td>Brand offer 10% higher price instead of 50% initially</td>
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<td>Contrast media</td>
<td>5–12%</td>
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Medication Cost Restriction in Aretaieio University Hospital, Athens Greece, 2011

Surgical – Obstetrics, Gynaecology, Paediatric – Radiology Departments

### O12 RESTRRAIN STRESS TECHNIQUES AND ULCEROGENICITY IN RATS

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Introduction: Restraining or immobilization procedure is a widely used laboratory technique for studying stress effects. A variety of restraint stress models have been developed for immobilizing animals in order to evaluate drug effects on stress-related pathology. Aim of the present study was to evaluate the formation of gastric ulcers in rats comparing four different rat restraint stress models. Materials and methods: 24 Wistar rats were divided into the saline and the pregabalin groups. All stomachs were removed and the numbers, as well as the total length of lesions were macroscopically counted. Furthermore, histological sections were assessed for vasoconges- tion, epithelial cell damage, inflammation and glandular disruption.

Results: Evaluating macroscopically the number and total length of lesions statistically significant differences were observed between the saline and the pregabalin groups. Microscopic examination revealed that vasocongestion and inflammation were also significantly reduced in the 30 mg/kg pregabalin group. Pregabalin inhibited stress-induced gastric ulceration in rats.

Discussion: Pregabalin inhibited stress-induced gastric ulceration in rats. Considering that its predominant mechanism of action is the inhibition of calcium currents via high-voltage-activated channels containing the a2d-1 subunit, the anti-ulcer effect results possibly from its anti-anxiety actions in combination with the sedative effects of the higher (30 mg/kg) drug dose.

### O13 EVALUATION OF THE ANTI-ULCEROGENIC ACTIVITY OF PREGABALIN IN RATS

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Introduction: It is well known that the central nervous system is intimately con- cerned in the genesis of stress-induced gastric ulceration. Restraining rats in a cold environment seems to be the most effective procedure for studying stress effects and for evaluating drug actions on stress-related pathology. Pregabalin is a derivative of the inhibitory neurotransmitter γ-aminobutyric acid and has been approved for treatment of seizures, different pain conditions, fibromyalgia and recently of generalized anxiety disorder. Aim of the present study was to evaluate the effects of this CNS active drug on stress-induced gastric ulcers. Materials and methods: 18 Wistar rats were divided into the saline and the pregabalin groups. All rats were subjected to a three-hour restraint procedure in a cold environment (4 °C). Pregabalin was dissolved in normal saline and administered intraperitoneally (i.p.) at doses of 15 ml/kg and 30 ml/kg. The drug was given 30 min before restraining the rats. The pretreat- ment times, route of administration and doses were taken from previous studies. After the restraint procedure each rat remained for two hours in his cage and then was sacrificed. The stomach was removed and the numbers, as well as the total length of lesions were macroscopically counted. Further- more, histological sections were assessed for vasocongestion, epithelial cell damage, inflammation and glandular disruption.

Results: Evaluating macroscopically the number and total length of lesions statistically significant differences were observed between the saline and the 30 mg/kg pregabalin groups. Microscopic examination revealed that vasocongestion and inflammation were also significantly reduced in the 30 mg/kg pregabalin group, whereas no difference was found in epithelial cell damage. Glandular disruption was observed only in one rat from each group.

Discussion: Pregabalin inhibited stress-induced gastric ulceration in rats. Considering that its predominant mechanism of action is the inhibition of calcium currents via high-voltage-activated channels containing the a2d-1 subunit, the anti-ulcer effect results possibly from its anti-anxiety actions in combination with the sedative effects of the higher (30 mg/kg) drug dose.

### O14 ESTROGENS DERIVED FROM THE GONADS AND THE BRAIN MEDIATE BEHAVIORAL RESPONSES DURING A TEST OF ANTIDEPRESSANT RESPONSE

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Women are more prone to depression than men and may have a differen- tial response to antidepressants, but tests for antidepressant screening have been mainly validated on male animals (Dalla et al 2010). In the present study, we applied the Forced Swim Test (FST), which is widely used for screening for antidepressant activity. During FST, rats are forced to swim in a cylinder for 15 min and the next day for 5 min. Previously, we have shown that female rats are particularly responsive to this test, but the role of estrogens in the behavioral profile during FST is not clear. Thus, in the present study, we tested females in all phases of the estrous cycle (all combinations of proestrous, estrous, diestrous 1 and 2) and we administered either vehicle or the SSRI sertraline in two doses (10 and 40 mg/kg, 3 i.p. injections). In a second experiment, we aimed to investigate the role of...
estrogens derived from neuronal sources, locally synthesized in the brain. Therefore, FST was performed 4 weeks after ovariectomy and castration of female and male rats respectively, in order to eliminate gonadal hormone secretion. Before the FST, rats were injected for one week with either vehicle or the aromatase inhibitor letrozole, which decreases estrogens synthesis in all tissues including the brain. In the first experiment, swimming duration, which is an active behavior, indicative of energetic activity, was lower when estrogens were also lower. Furthermore, this behavior positively correlated with the estrogen-dependent uterus weight. Sertraline treatment exerted an antidepressant effect by enhancing swimming and decreasing immobility in males and females in all phases of the estrous cycle. In the second experiment, letrozole-inhibited induction of estrogens in ovariectomized females enhanced immobility and decreased active behavioral responses, which is indicative of enhanced “depressive-like” symptomatology. On the contrary, it had no effect on males. These results indicate that estrogens originating from the gonads and the brain significantly affect the FST behavioral response. However, the phase of the estrous cycle does not influence the antidepressant response. Our data suggest a role of estrogen-inhibition in the development of affective disorders in women treated with aromatase inhibitors.

O15
THE EFFECTS OF SIBUTRAMINE ON SERUM GHRELIN ISOFORMS AND PARAVENTRICULAR NUCLEUS NPY CONCENTRATIONS IN RATS UNDER THREE ISOCALORIC DIETS
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Introduction: Appetite regulation is a complex process that involves both central and peripheral signals. Among orexigenic peptides, the most potent is neuropeptide Y (NPY) and ghrelin. The hypothalamus is the main regulatory organ for the human appetite and energy balance. Since the neurons that regulate appetite appear to be mainly serotonergic and noradrenergic, pharmacological agents targeting these neurotransmitters or their receptors could modulate food intake and body weight.

Aim: The aim of the study was to investigate: a) The differential effects of macronutrients on food intake, fasting serum ghrelin, ghrelin isoform distribution and paraventricular nucleus (PVN) NPY b) The impact of sibutramine (S), which is a serotonin-norepinephrine reuptake inhibitor, on the aforementioned parameters in rats fed ad libitum with three isocaloric diets.

Methods: Three groups of male Wistar rats (n=63) were fed with high fat diet (HFD) (n=21), high carbohydrate diet (HCD) (n=21), or high protein diet (HPD) (n=21) for 13 weeks. In the last 3 weeks each group was divided into 3 subgroups and received intraperitoneally S 5mg/kg, S 10mg/kg or saline vehicle. Food intake was measured daily during the last week of the experiment. The PVN was isolated from the hypothalamus and NPY was measured. Serum desacylated and acylated ghrelin and PVN NPY levels were assayed.

Results: HFD fed rats demonstrated increased food intake and PNV NPY content. Serum desacylated ghrelin levels were significantly higher in the HCD group compared to any other group. S at 10mg/Kg decreased food intake in the HFD fed rats and tended to increase fasting serum desacylated ghrelin, without affecting acylated ghrelin or NPY.

Conclusions: Results suggest a role of NPY in HFD-mediated hyperphagia. On the other hand, neither NPY, nor acylated ghrelin seem to be involved in the anorectic effects of sibutramine. Desacylated ghrelin was lower in rats under HFD, although the effects of macronutrients could not be dissociated from those of adiposity. A trend towards elevated desacylated ghrelin levels in the HCD compared to the HPD subgroup was also observed, a fact that requires further investigation.

O16
CHOLINE DEFICIENCY MODULATES MYOCARDIAL AUTO-NOMIC NEUROTRANSMISSION IN THE RAT: THE EFFECT OF CARNITINE
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Introduction: Choline belongs to the B group of vitamins and is considered an essential nutrient with lipotropic properties. A significant interaction between streptozotocin (STZ) induced diabetes and choline metabolism has been reported, although insulin’s effects in the liver is not fully elucidated. Glut-4, although expressed in adipose tissues and striated muscle. GLUT-4 is expressed primarily in muscle and fat cells, the major tissues in the body that respond to insulin. When an insulin receptor is activated, it induces the GLUT-4 protein to move from intracellular vesicles to the cell membrane, allowing glucose to enter the cell. This process is known as insulin-mediated glucose transport.

Aim: The aim of this study was to investigate the effect of choline deficiency on the modulation of rat liver activity of GLUT-4 caused by adult-onset streptozotocin (STZ)-induced diabetes.

Materials and Methods: Male Wistar rats (n=48) were divided in four groups: a) control (C), b) rats receiving choline deficient diet (CDD), c) diabetic rats receiving balanced diet (D) and d) diabetic rats fed with choline deficient diet (D + CDD). The duration of the experiment was 4 weeks.

Results: GLUT-4 expression was increased by 550.43% in the CDD group, by 168% in the D group and by 300.5% in the D+CDD group compared to the control (p<0.001).

Conclusions: There is an up-regulation of the GLUT-4 activity in all groups and mostly in the CDD group due to the fatty infiltration caused by the choline deficiency. These data show crucial alterations to the insulin pathway and further studies are in progress in order to investigate underlying mechanisms.
P1
PHARMACOGENOMIC ANALYSIS OF THE ATEROPROTECTIVE ROLE OF APOE3 CONTAINING HDL IN HUMAN ENDOTHELIAL CELLS
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Introduction: Apolipoprotein apoE3 contributes to atheroprotection in multiple ways. Specifically, it facilitates lipoprotein particle hepatic uptake and contributes to cholesterol homostasis in the plasma. Furthermore, it promotes the synthesis of HDL particles that contain apoE (HDL-apoE), and are thought to contribute to endothelial function. Lack of apoE3 in mice has been shown to lead to premature atherosclerosis. The aim of our study is the characterization of the molecular mechanisms affected by endothelial cell exposure to recombinant HDL containing apoE3 and phospholipids (HDL-apoE3).

Methodology: Primary human arterial endothelial cells (HAEC) were exposed to HDL-apoE3 or PBS. Isolated RNA was labeled and hybridized to whole genome microarrays (28,869 genes), namely GeneChip Human Gene 1.0 ST Array (Affymetrix). Five samples/microarrays were used for treatment. The raw data were submitted to extensive bioinformatical analysis using 2-fold and ≤0.05 false discovery rate thresholds. A total of 198 genes were detected after ImageJ-analysis. Neuronal differentiation was evaluated by alterations of synaptophysin and NCAM protein levels before and after agonist treatment using specific antibodies generously provided by Dr M. Gaitanou, Hellenic Pasteur Institute, Athens [9].

Results-Discussion: To examine the effect of δ-OR-induced STAT5B activation on differentiation and neurite outgrowth, Neuro-2A cells were treated with the δ-opioid agonist DSLET and a) the levels of NCAM, synaptophysin were detected after immunoblotting and b) by measurements of neurite length. Our results have shown that DSLET administration resulted in increased neurite outgrowth, which was blocked by pertussis toxin pre-treatment and the expression of a dominant negative STAT5B (DN-STAT5B) mutant. Additional studies have shown that while DSLET exposure of Neuroblastoma cells induced a marked increase of synaptophysin and NCAM protein levels, overexpression of the DN-STAT5B resulted in a profound decrease in the expression of both protein levels. Taken together, our findings demonstrate that δ-OR activation leads to neuronal differentiation and neurite outgrowth via a signaling pathway involving Gαi/o proteins and STAT5B.

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References

P2
ACTIVATION OF THE δ-OPIOID RECEPTOR LEADS TO DIFFERENTIATION AND NEURITE OUTGROWTH VIA A STAT5B-GAI/O SIGNALING PATHWAY
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Introduction: Neurite outgrowth is a key process during neuronal migration and differentiation. Complex intracellular signaling mechanisms are involved in the initiation of neurite protrusion and subsequent elongation. Opioid receptors couple to Gi/Go proteins and participate in various cellular mechanisms controlling neural growth, differentiation and synaptic plasticity [1-3]. We have recently demonstrated that δ- and μ-opioid receptors (δ-OR and μ-OR) form multi-component signaling complexes, consisting of the Signal Transducers and Activators of Transcription 5A/B (STAT5A/B), e-Src kinase and selective G protein subunits, leading to STAT5A/B phosphorylation [4,5]. We were thus interested whether δ-opioid receptor present in Neuro-2A cells triggers differentiation and neurite outgrowth through activation of a signalling network involving STAT5B, members of Gi/Go proteins and Src.

Methodology: Cell cultures and transient transfections: 293F or HEK293 cells stably expressing the human μ-opioid receptor (μ-OR) or the flag-δ-OR respectively were grown and transiently transfected with either pCDNA3 or the cDNAs of B/R4-RGS family to interact with kappa (κ-OR) and δ-OR. Protein-protein interactions were detected using co-immunoprecipitation assays, and in vitro pull down assays using GST-fusion peptides encompassing the δ third intracellular loop (δ-3L), δ-CT and κ-CT but not with μ-CT. Co-immunoprecipitation experiments using cell lysates co-expressing κ-OR and δ-OR along with HA-RGS2 or HA-RGS4 indicated that both receptors interact with RGS2 and RGS4 proteins constitutively and upon agonist stimulation.

Functional assays in cells expressing either RGS2 or RGS4 displayed a differential regulatory effect on δ-OR and κ-OR signaling suggesting that
although these receptors interact with the same subsets of RGS proteins each of them affects signaling in a distinct manner. Collectively, our results suggest that RGS-oxidop receptor pairs may be influenced by different factors depending on: a) the level of receptor expression, b) the activation state of the receptor and c) the abundance of the G protein present in a cellular milieu.

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References

P4
STRUCTURE-FUNCTION ANALYSIS OF THE THIRD MEMBRANE-SPANNING SEGMENT OF CRF1
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Corticotropin releasing factor (CRF) exerts most of its physiological and pathophysiological actions by interacting with its type 1 receptor (CRF1). CRF1 consists of seven plasma membrane-spanning segments (TM1-TM7), which have been recently shown by our group to form a water-accessible crevice, which extends from the extracellular surface of the receptor into the plane of the membrane. Among the TM residues of CRF, His199 in the TM3 has been proposed to play role in non-peptide ligand binding. This leads to the hypothesis that His199 and/or other TM residues are located on the surface of the binding-site crevice of CRF, and interact with non-peptide ligands, such as antalarmin. However, the lack of information about the structure of TM3 and the precise interactions of their residues with ligands precludes the assessment of this hypothesis. To test this hypothesis we mutated His199 to Ala and determined antalarmin affinity before and after mutation. We found that His199Ala mutation did not significantly decrease antalarmin affinity, suggesting that His199 did not interact with antalarmin. To elucidate the role of other TM3 residues of CRF, in the binding of non-peptide analogues we must first determine the amino acids that are located on the surface of the binding-site crevice of CRF, we applied the cysteine-substituted accessibility method (SCAM), using as background the ΔCys mutant of CRF1, and starting from the extracellular portion of TM3. The ΔCys mutants has near normal functional properties and it is relatively insensitive to the positively charged sulphydryl-specific reagent, MTSEA. We mutated ten TM3 residues of CRF, to Cys, one at a time. Five of these mutants, Thr192-Cys, Ala193Cys, Tyr195Cys, Asn196Cys and His199Cys reacted with the MTSEA, added extracellularly. We therefore suggest that the side chains of residues at the reactive loci (Thr192, Ala193, Tyr195, Asn196, and His199) are on the water-accessible surface of the binding-site crevice of CRF. The pattern of accessibility is consistent with an alpha-helical conformation for this segment of CRF1.
Discussion: Glutamate, a major neurotransmitter in CNS, in many neurodegenerative diseases can act as a neurotoxin leading to excitotoxicity and increased intracellular calcium influx. The role of the Grp family (chaperones), in cell survival or cell death under excitotoxic conditions is a field of intense research. Unraveling the underlying molecular mechanisms can provide insight into new targets for pharmacological treatment on bone mineral density and bone formation-resorption markers in streptozocin-induced diabetic rats.

Methods: Ten-week-old male Wistar rats were divided into 4 groups: non-diabetic controls, control rats receiving pioglitazone (3 mg/kg), streptozocin-treated diabetic rats (50 mg/kg), and diabetic rats treated with pioglitazone (3 mg/kg). The duration of the experiment was 8 weeks. Small animal high-resolution scan was performed (line spacing 0.3 mm) using a Hologic Discovery (Bedford, MA, USA). A mean value of bone mineral density (BMD-gram/cm²) for the whole left femur and two sub-regions, the diaphysis and proximal metaphysis was measured.

Results: Diabetes in our rats was associated with weight loss. Diabetic rats had reduced plasma osteocalcin levels and increased calcium excretion in the urines. Regardless of the studied site, there was no significant difference in bone mineral density between the four groups.

Conclusion: Pioglitazone administration at the 3 mg/kg dose had no impact on bone formation and resorption markers levels and did not modify bone mineral density in our experimental model.
Osram Ultra-Vitalux® light bulb with an emission radiation spectrum similar to that of normal sunlight at noon, and exposed for 30 min to an equivalent of 7.500 J/m² of UVB irradiation, in the range of the reported threshold for corneal damage. Corneal and conjunctival tissue samples were removed from exposed eyes at 2, 6 and 24 hours following the end of the exposure to the bulb light, and were subsequently processed for histochemical staining and DNA extraction. The gene expression of tumor necrosis factor (TNF), interleukin 6 (IL-6) and PAFR was monitored with conventional RT-PCR.

Rupatadine, dissolved in DMSO, was applied topically in concentrations similar to those routinely used in oculoplastic preparations of other antiglaucomatous drugs, one hour before, immediately after, and one hour following exposure.

Results: No specific alterations were detected, using standard eosin-hema-toxylin staining, in corneal tissue, as a result of acute exposure to artificial sunlight. In the conjunctiva however, a marked accumulation of eosinophils was noticed, as early as hour 2 post-exposure, which appears to be directed towards the upper part of the epithelial layer. This effect appears to subside by hour 24. No statistically significant changes were detected with respect to the gene expression examined, in either tissue. Rupatadine did not affect eosinophil accumulation in the conjunctiva or the gene expression in either tissue.

Conclusion: Acute exposure to artificial sunlight causes an accumulation of eosinophils in rabbit conjunctival epithelium, which was not prevented by the topical application of rupatadine, under the conditions used in this study.

P11 NEW SYNTHETIC COUMARIN DERIVATIVES WITH POTENT ANTI-INFLAMMATORY, HYPOCHOLESTEROLEMIC AND ANTIITHROMBOTIC ACTIVITY

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It has been reported that inflammation can have an important role in the initiation, progression of cardiovascular diseases (CVDs) and blood coagulation cascade. The designation of CVD as a chronic inflammatory process is further supported by evidence that the risk factors for CVD cause endothelial cells throughout the vascular tree to assume an inflammatory phenotype. These activated endothelial cells characteristically exhibit oxidative stress and increased adhesiveness for circulating leukocytes. Although initial efforts to define the mechanisms underlying the inflammatory phenotype in diseased endothelial cells have focused on the linkage between oxidative stress and adhesion molecule activation/expression, recent work has implicated a variety of additional factors that can modulate the magnitude and/or nature of the inflammatory responses in CVD. Activation of blood coagulation and thrombin formation accompany inflammation, wound healing, atherosclerosis and other processes induced by endothelial injury.

Coumarins comprise a large class of phenolic substances occurring in plants. Coumarins’ natural and synthetic derivatives were found to possess significant anti-inflammatory and antioxidant activities. It is well known that coumarin derivatives, both natural and synthetic, have been studied for long. Many series of coumarin derivatives which have been studied for their in vivo anti-inflammatory activity, using the carrageenan induced rat paw edema model, have been presented from our research group. Many coumarin compounds are recognized as lipoygenase and cyclooxygenase inhibitors. In this study we tested the effect of two coumarin Mannich bases, designed and synthesized as potent anti-inflammatory and antioxidant agent, on several inflammatory indices in male cholesterol-loaded (feeding with 2% cholesterol and 6% corn oil for 120 days) atherosclerotic NZW rabbits. Blood samples for lipids and anti-inflammatory indices [C3, C4, CRP, IL-1α-antitrypsin (AAT), haptoglobin (HAT)] were taken before and after feeding as well as after the 7-day administration of substances K12 and K13 Results were analyzed by the Friedman’s rank test. In general the treatment with the tested compound induced significant decreases (p<0.05) of the values of C and AAT. Values of all other indices tended to decrease without significant difference. It is concluded that the tested compound shows satisfactory results and it must be investigated thoroughly.

P12 CHANGES IN BLOOD PARAMETERS AFTER ADMINISTRATION OF A NEW SYNTHESIZED-INFLAMMATORY ANTIOXIDANT AGENT IN ADULT RATS AFTER SCIATIC NERVE CRUSH

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Introduction: The EP-B11 is essentially an anti-inflammatory and antioxidant compound which has been designed, synthesized and biologically evaluated in the Department of Pharmaceutical Chemistry of Aristotle University of Thessaloniki in the process of a thesis (E. Pontiki, 2007) and afterwards was further developed and studied in the laboratory of the Department of Clinical Pharmacology. The compound displayed: a) anti-inflammatory activity in vivo by inhibiting the onset of inflammation (edema) induced after the intradermal administration of carrageenin into the right foot pad of Fisher rats and b) significant antioxidant behavior in various in vitro experimental protocols. It was considered that the next logical step should be to study the behavior of this agent experimentally in the case of peripheral injury and specifically in the case of the sciatic nerve injury using the method of nerve crush in rats. The injury is achieved by crushing with forceps the sciatic nerve, a procedure that is known to activate the mechanisms of inflammation which delay the regeneration of nerve.

Methods: We investigated the effect of EP-B11 agent in haematological parameters following peripheral sciatic nerve injury in adult rats. The study was included the following: a) control group in which no EP-B11 agent was administered and b) two experimental groups (1 and 2) in which the examined compound was administered from the first day of injury. Animals in groups 1 and 2 were sacrificed on the first and second day after injury respectively. After stunning them with chloral hydrate at a dosage according to their body weight, a blood sample from the left atrium was taken. In the 3 groups the following haematological parameters were identified and measured: hematocrit, white blood cells, granulocytes, platelets, PT, PTT, fibrinogen, alpha 1-antitrypsin, ESR, CRP and they then were analyzed statistically using the statistical package SPSS.

Results: It is observed a statistically significant high increase in the number of white blood cells and a decrease in platelet count in group 2 (animals sacrificed on the second day after injury where the agent was administered) compared with the other groups, while the ESR and CRP were not mobilized. The increased number of white cells probably reflects the evolution of the inflammatory process in injured rats and leads to the logical expansion of the experiment in further time points after injury.

P13 CONSUMPTION OF ENERGY DRINKS BY STUDENTS ARISTO-LE’S UNIVERSITY OF THESSALONIKI. FIELD RESEARCH

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Energy drinks are a special category of beverages which even though they are very popular, only a little is known about them. The level of knowledge about energy drinks in Greece is unknown for both public and scientific community of health professionals.

This research aims to reveal the grade of consumption of energy drinks and the tension of people to combine them with alcohol. For this purpose a questionnaire was used. The target group was students from Aristotle’s University of Thessaloniki regardless of age, sex and school. From the sample of 300...
students which were randomly chosen, 270 students remained as subjects. The results showed that 47% of the students consume energy drinks and 75.6% declared that they combine them with alcoholic beverages and 75.6% agreed with the opinion that this combination could harm health. Men tend to consume energy drinks in higher grade than women (men/women=68/59) but this relationship is not strong (p=0.057). On the contrary, there is a strong relationship between school and consumption of energy drinks (p=0.02). Gymnastics Academy collects the highest proportion (67%) in consumption of energy drinks among other schools. The main reason of energy drink consumption is the need for energy. The instability and the variety in student’s opinion about energy drinks reveal the lack of information or misinformation about them. Energy drinks could be dangerous when they are combined with alcohol mainly because this combination could cause dehydration. The interaction of energy drinks with alcohol depends on dose and individual’s sensitivity. At low doses of ethanol caffeine, which is contained in energy drinks, also reduces the depressant effects of ethanol. In high doses of ethanol the ingestion of energy drinks reduces the intensity of some subjective symptoms of alcoholic intoxication but does not reduce the deficits because of alcohol ingestion, evaluated by objective tests such as motor coordination and visual reaction time.

The fact that almost half of students consume energy drinks leads to the necessity of information about their probable benefits and their side effects. Sensible consumption of energy drinks is a matter of public health and both health professionals and public should be informed about them.

P14
PHARMACOLOGICAL TREATMENT OF GENITAL LICHEN SCLEROSUS- A REVIEW.
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Purpose: This is a review of the literature about the pharmaceutical management of genital lichen sclerosis, which affects men and women of all ages.
Materials and methods: We performed a comprehensive search of the literature in PubMed and other electronic databases between 1990 and 2012 using the key words genital lichen sclerosus, lichen sclerosus atrophicus, topical treatment, balanitis xerotica and randomized control trials.
Results: Lichen sclerosus (LS) is a chronic, lymphocyte-mediated skin condition of uncertain etiology. The main purpose of pharmaceutical treatment is to improve the symptoms, which in many cases are persistent and irritating. In addition, limiting the relapses is important since squamous cell carcinoma (SCC) has been associated with anogenital LS. The topical therapy for LS includes: topical corticosteroids, hormones, calcineurin inhibitors, antihistamines, and antipruritic agents. acetatin, cyclosporine, antibiotics and retinoids have been used for the systemic therapy of LS.
Conclusion: Due to the elevated risk of malignancy and in order to improve quality of life, all patients with LS should receive treatment. Many therapies have been used with uncertain outcomes and in these cases biopsy should be recommended. Further research on new pharmacologic agents could provide a better prognosis.

P15
PPARα/γ AGONISTS: A NOVEL APPROACH TO ANTI-DIABETIC THERAPY
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Introduction: Recently, a novel therapeutic approach against type 2 diabetes mellitus (DM) using dual peroxisome proliferator-activated receptor α/γ (PPARα/γ) agonists is under thorough scientific research. It is thought that combination of the actions of fibrates and thiazolidinediones will not only cure DM but also prevent the occurrence of diabetic dyslipidemia and the future development of macrovascular diabetic complications. Methods: PubMed and Scopus were the data sources of our study. We used the terms “PPAR α/γ OR PPAR alpha/gamma” and the limitation of “2010 to present” at publication year. Two independent reviewers screened 17 (PubMed) and 41 (Scopus) articles for relative abstracts or titles. After excluding duplicates, 34 articles which consisted the material of our study, were identified. Results: Novel thiopephosphate substituted oxazole containing α-alkoxy-

phenylpropanoic acid derivatives (glitazars) act as dual PPARα/γ agonists. Generally, glitazars reduce on the one hand hyperglycemia by ameliorating insulin resistance and on the other hand dyslipidemia by modifying patient’s HDL and triglyceride profile. PPARα/γ agonists are under clinical trials following studies in rodents, concerning mainly DM but also cardiovascular disease and obesity-related disorders. However, many of the trials of these compounds discontinued due to side effects, such as increased weight gain and serum creatinine levels, peripheral edema, myocardiocirfarction or stroke and in some cases of the studies in rodents, discontinued due to their potential carcinogenicity.

Conclusion: The effective treatment of type 2 DM in combination with the prevention of diabetic macrovascular complications which dual PPARα/γ agonists promise is undoubtedly an important issue. However, due to their side effects, new selective partial agonists should be identified in order to use their therapeutic actions in an efficient and safe way.

P16
ARSENIC TRIOXIDE. A MODERN ‘WEAPON’ FROM THE PAST AGAINST ACUTE PROMYELOCYTIC LEUKEMIA
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Acute Promyelocytic Leukemia (APL) has become the most curable of all leukemias since Arsenic Trioxide (ATO) was introduced. As a single agent or in combination with all-trans retinoic acid (ATRa) is recommended by the European LeukemiaNet guidelines as a first option for relapsed patients. However, the role of ATO as a single agent is still under consideration because there are only a few trials which support its beneficial action as a single therapy. Arsenic is a well-known poison that can be used as a medicine and this was familiar to early physicians such as Hippocrates. Its narrow therapeutic dose and the side effects coming from its toxicity are the two reasons for which medical society is still skeptical about its applications in oncology. In this article are discussed the latest evidence from the international literature about the benefits of using ATO in combination with other drugs and its value as a single agent in the struggle against APL. Its low price and the controlled side effects give a promising option to the use of APL as a first-line drug in APL. However, future studies, especially clinical trials, must be designed in order to have evidence based use of ATO in APL.

P17
INFLUENZA H1N1 PROPHYLAXIS FOR EXPOSED NEONATES USING OSELTAMIVIR
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Introduction: Despite the fact that influenza vaccination during pregnancy is recommended to protect both mothers and fetus, young infants are at increased risk for serious illness, development of complications, and hospitalization following influenza infection, including influenza H1N1 pneumonia. However, influenza vaccine is not licensed for infants younger than six months and vaccination, the main measure for the prevention of influenza, rates among pregnant women remain rather low. Therefore, the prevention of spread of influenza to neonates within a NICU relies on strict implementation of infection control measures along with antiviral post-exposure prophylaxis. Thirteen neonates hospitalized in the Aghia Sophia Children’s Hospital NICU in Athens were exposed to pandemic influenza H1N1. Hereby, we present the serum pharmacokinetic data of oseltamivir prophylaxis administered at the dose of 1.0 ml/kg b.i.d., and specifically the determination of the conversion rate of oseltamivir.
phosphate to its principle metabolite oseltamivir carboxylate.

Methods: We measured pharmacokinetics of oseltamivir prophylaxis at 1.0 mg/kg b.i.d. x 10 days given to 13 neonates (median age: 15 days; median weight: 3565g) exposed to H1N1. Plasma samples were analyzed with a modified LC-MS/MS protocol. Data analysis was performed using the NONMEM technique. Results: All neonates completed their 10-day course (20 doses each). All but one received antibacterial treatment concomitantly; no other medications were administered. None of the neonates developed influenza during their follow-up. Four developed diarrhoea. No neurologic or laboratory adverse effects occurred. Mean Cmax concentrations (± SD) for oseltamivir (9.38 ± 4.50 ng/mL) and oseltamivir carboxylate (65.62 ± 32.31 ng/mL) were lower than those reported in children 1-5 years. Tmax values for oseltamivir (1 h) and oseltamivir carboxylate (4 h) agreed with those in older groups. Conclusions: Our data showed that as the age of the neonate increases oseltamivir clearance diminishes. Furthermore, newborn females had a higher ability to clear oseltamivir more rapidly than newborn boys. Neonates metabolize Oseltamivir and the dose of 1.0 mg/kg b.i.d. for 10 days appears to be safe for prophylaxis against influenza.

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VISUAL ANALOG SCORES' DEPENDENCY FROM IL-6 AND CRP IN THORASIC SURGICAL PATIENTS

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Background/Aim: Aim of the study was to investigate the possible regression of visual analog scores (VAS) of postoperative lung cancer patients from Interleukin-6 (IL-6) and C-reactive protein (CRP).

Material/Methods: Eighteen patients undergoing thoracic surgery were evaluated (as part of a relative Thesis) sequentially in the postoperative period (four days) with VAS scores, in immobility and movement/cough. Also, measurements of CRP and IL-6 were performed at the same time and day. Multiple linear regression was used (Statistical software of Microsoft Office Excel 2007) and a=0.05 was considered as statistically significant. Results: In n=72 pairs, when response variable was VAS in stillness or in movement, adjusted R2 was 0.29 and 0.55, respectively. In VAS stillness, mean Cmax concentrations (± SD) for oseltamivir (9.38 ± 4.50 ng/mL) and oseltamivir carboxylate (65.62 ± 32.31 ng/mL) were lower than those reported in children 1-5 years. Tmax values for oseltamivir (1 h) and oseltamivir carboxylate (4 h) agreed with those in older groups. Conclusions: Our data showed that as the age of the neonate increases oseltamivir clearance diminishes. Furthermore, newborn females had a higher ability to clear oseltamivir more rapidly than newborn boys. Neonates metabolize Oseltamivir and the dose of 1.0 mg/kg b.i.d. for 10 days appears to be safe for prophylaxis against influenza.

P19

NECK PAIN'S ASSOCIATIONS TO STRESS BIOMARKERS

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Abstract: Pain has been shown to be associated with stress-related disorders. Physiological and psychological mechanisms have been proposed to link stress to musculoskeletal pain and a number of stress biomarkers in patients with chronic pain.

As mechanical cervical nerve root irritation can be a source of neck pain, spinal neuropeptides can mediate pain responses. Peptides released in the spinal cord from the central terminals of nociceptors contribute to the persistent hyperalgesia that defines the clinical experience of chronic pain. The presence of substance P and calcitonin gene-related peptide reactive nerve fibers in a population of these linds cede to cervical facet joint capsules as a key source of neck pain. Salivary cortisol is also a useful biomarker in stress research, as long as the researcher is aware of possible sources of variance, which may affect this measure. Other stress biomarkers are S-DHEA-S and P-endothelin, S-insulin and P-fibrinogen. Longitudinal analysis of changes in pain levels and stress biomarkers within an interval of 6 months showed beneficial changes in the following stress markers: P-NPY, S-albumin, S-growth hormone and S-HDL when pain decreased, and vice versa when pain increased. Stress biomarkers with predicting value for pain are S-DHEA-S and S-albumin and higher B-HbA1c and P-fibrinogen. These findings might contribute to increased knowledge about strategies to prevent further progression of neck/shoulder/back pain in persons who are “not yet in chronic pain”. Because of the complex interactions that exist between stress and the activation of the HPA axis, it is important that careful consideration be given to the best experimental design for each investigation. This research indicates that stress biomarkers through a variety of biochemical pathways can be used to predict and manage pain in future. Key words: stress biomarkers, neck pain, p-substance, s-cortisol, chronic pain.

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GROWTH-HORMONE-RELEASING HORMONE RECEPTOR SUBTYPES IN BREAST CANCER

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Objective: The hypothalamic neuropeptide GHRH, upon binding to specific receptors (pGHRH-R), stimulates the synthesis and release of GH from the pituitary. pGHRH is also found in extrahypothalamic tissues, including neoplasms. Splice variant 1 of GHRH receptor (SV1) is widely expressed in non-pituitary tissues and cancers. Accumulated evidence implies several roles for pGHRH-R and SV1 in carcinogenesis. The aim of the present study was to investigate the expression of pGHRH-R and SV1 in human breast tumors and to correlate the results with the histo/clinicopathological characteristics of the patients, their clinical course and survival.

Design: Receptor expression was studied in 33 breast biopsies from patients diagnosed with primary breast adenocarcinoma, obtained from the tumor and the adjacent tissue.

Methods: pGHRH-R and SV1 gene expression levels were evaluated by real-time PCR following reverse transcription of total RNA extracts. Data were analyzed by SPSS.

Results: pGHRH-R was found in 50.0% of malignant and 53.8% of benign biopsies. SV1 was found in 37.0% of malignant and 29.6% of benign biopsies. Transcript levels of pGHRH-R were 4.189554±10.4429 in malignant and 10.1286±19.53721 in benign biopsies, whereas the respective levels of SV1 were 1.415537±0.02818 and 1.736±0.102704. Statistical analysis revealed no differences in rate and levels of expression between benign and malignant biopsies, as well as between the expression of pGHRH-R and SV1 in malignant tissues. Correlation analysis with the demographic and clinical characteristics of the patients and the histopathological characteristics of the tumors showed a positive correlation between transcript levels of SV1 and height, whereas the statistical analysis between the expression of the receptors and the recurrence of the disease or the survival of the patients revealed no significant differences.

Conclusions: pGHRH-R and SV1 were found in breast tumors and adjacent tissue. Transcript levels did not differ between them in a statistically significant manner, implying no overexpression by the tumor cells. Receptor expression did not correlate to any of the patient and tumor characteristics, except height, a known risk factor for breast cancer. No correlation was also found with disease recurrence and the patient survival. Further studies are needed in order to unfold biological effects mediated by GHRH receptors in breast cancer and their potential as therapeutic targets.

P21

PHENOTYPIC AND GENOTYPIC ANALYSIS OF CYP1A2 IN THE GREEK POPULATION

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Introduction: CYP1A2 is a key enzyme for the metabolism of many clinically used drugs and the activation of procarcinogens. Its activity can be modulated by dietary and environmental factors. Several single nucleotide polymorphisms
The CYP1A2 gene has been reported to affect enzyme inducibility. In the present study we investigated the distribution of two of the most common SNPs in the 5‘-flanking region and in intron 1 of CYP1A2 gene in the Greek population in parallel with CYP1A2 phenotypic activity.

Methods: Spot urine samples of 44 healthy women were analyzed 6 hours after 200 mg caffeine intake following a 24-hour xanthine-free diet. Phenotypic analysis of CYP1A2 activity was accomplished by estimating the caffeine metabolic ratio (AFMU:1+131I/17U) using RP-HPLC method. DNA isolated from peripheral blood samples was genotyped for -3860 G/A (allele CYP1A2*1C) and -163 C>A (allele CYP1A2*1F) polymorphisms by PCR-RFLP method.

Results: Median values (range) of the metabolic ratio were 4.44 (2.42-9.18) and 3.05 (1.81-6.32) for smokers (n=19) and non-smokers (n=25), respectively (p=0.001). Frequencies for CYP1A2*1F polymorphism were 25/44 (56.8%), 18/44 (40.9%) and 1/44 (2.3%) for the C/A, A/A and C/C genotypes, respectively, whereas the G>A polymorphism in -3860 position was not detected. Smokers with the A/A genotype tended to have higher median metabolic ratio than C/A carriers (5.08 versus 3.92, p=0.107), while no such difference was noticed in non-smokers (3.15 versus 3.03, p=0.977). Conclusion: CYP1A2 polymorphism -163 C>A is widely distributed in Greek females and may provide useful information regarding the inter-rate of inactivation of CYP1A2 substrates, such as prescribed drugs. In conclusion, the CYP1A2 polymorphism displays, in our study, an effect which may be attributed to the small sample size.
Discussion: The statistical analysis showed that throughout the study population, wild type genes actually occur at a greater rate than the mutant ones. The genes’ distribution between men and women is not significantly different. We could not draw conclusions regarding the correlation of the studied polymorphisms with AD, as we do not know if the people involved were healthy or not. However, knowledge of the genetic background will help in the constant effort to find effective treatment for AD, as knowing the gene mechanisms involved in its development, we can design new drugs aiming directly at them.

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HAPLOTYPE EVALUATION OF CATECHOL-O-METHYLTRANSFERASE ENZYME POLYMORPHISMS IN SCHIZOPHRENIA: A CASE-CONTROL STUDY IN A GREEK POPULATION

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Introduction: Schizophrenia, a severe psychiatric condition, characterized by disturbances of cognition, emotion and social functioning, affects almost 1% of world population. It has been assumed that schizophrenia occurs as a result of a primary defect within the dopamine neurotransmission system.

Aim: Recent studies that evaluated the role of Catechol-O-methyltransferase enzyme (COMT) polymorphisms in the occurrence of schizophrenia have resulted in ambiguous findings. The current study was conducted in order to gain an insight on the possible association of schizophrenia with three polymorphisms, namely, rs737865, rs4680 and rs165599.

Materials and methods: All schizophrenia patients participating in the study were recruited from the Athens “Dafni” Hospital whereas controls from the sample bank of the spin off company of University of Athens “Research Diagnostics”. SNPs were genotyped using PCR-real time analysis. Chi-square test and logistic regression analysis were used to assess differences among cases and controls.

Results: A total of 108 patients diagnosed with schizophrenia and 97 individuals without a history of psychiatric disorder participated at the study. None of the three SNPs rs737865, rs4680 and rs165599 were found to be independently associated with schizophrenia. However, haplotype analysis showed that cases have higher expression of the T-A-A haplotype and lower frequency of the T-G-G haplotype. Participants with the T-A-A haplotype were at increased risk for developing the disease (OR=1.52; 95% CI: 1.12-2.08; p=0.008). Similarly, participants with T/T-A/A-A/A were more susceptible to the disease (OR=2.13; 95% CI: 1.02-4.47; p=0.045). Regarding T/G/G, we found a protective effect of T/T-G/G-G/G (OR=0.22; 95% CI: 0.09-0.56; p=0.001) and T/T-G/A-G/G (OR=0.33; 95% CI: 0.12-0.87; p=0.025)

Discussion: In our haplotype analysis, we found that the A-A-A haplotype had association with the disease. Bray et al, showed that G-G-G (C-G-G) haplotype is associated with low COMT mRNA expression in prefrontal cortex. It is possible that the opposite haplotype A-A-A (T-A-A), which is the risk haplotype in this study confers high COMT mRNA expression. This hypothesis is compatible with the reformulated theory of hypofrontality in schizophrenia. Our study shows an association of the COMT gene and schizophrenia in Greek population.

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PREVALENCE OF THE INSERTION/DELETION (I/D) POLYMORPHISM OF THE ACE GENE AND THE GLU298ASP POLYMORPHISM OF THE ENOS GENE IN A POPULATION OF GREEK PATIENTS WITH OR WITHOUT HYPERTENSION

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Introduction: Hypertension is a major risk factor for cardiovascular disease. Essential hypertension and its pathogenesis depend on a complex interaction of genetic and environmental factors. Endothelium-derived nitric oxide (NO), which is synthesized by endothelial nitric oxide synthase, plays an important role in the regulation of endothelial function and in the control of blood pressure. The Renin-Angiotensin-Aldosterone System (RAAS) is a well characterized mechanism for the regulation of blood pressure in which the Angiotensin Converting Enzyme (ACE) is one of the most important components. The role of the Glu298Asp polymorphism of the eNOS gene and the I/D polymorphism of the ACE gene in hypertension has been examined in a large number of studies with conflicting results. The objective of this study was to investigate the relationship between these two polymorphisms, separately and combined, with essential hypertension in a Greek population.

Materials and Methods: The study sample comprised of 200 participants. Among them, 104 were hypertensive patients and 96 healthy individuals who were used as controls. Genotyping for ACE polymorphism was performed with PCR, followed by electrophoresis and for eNOS polymorphism with real time PCR.

Results and Discussion: The groups of hypertensive patients and control subjects were age and sex matched. Regarding Glu298Asp polymorphism of the eNOS there was no significant difference in the contribution of alleles or genotypes among the groups. A higher percentage of DD genotype of the ACE I/D polymorphism (52.9% vs. 39.6%) and a lower percentage of ID genotype (27.9% vs. 44.8%) were observed in the group of hypertensive patients compared to the control group (p=0.044). Logistic regression did not show any association of the polymorphism with hypertension. Logistic regression performed with combinations of genotypes from these two polymorphisms, revealed that the carriers of the GT/DD genotypes had a 65% lower risk for hypertension compared to GG/DD genotypes carriers (OR=0.35; 95% CI: 0.13-0.89; p=0.028). Furthermore, the carriers of the GT/DD genotypes were found to have a 82% lower risk for hypertension (OR=0.18, 95% CI: 0.04-0.76, p=0.020) and the carriers of GG/DD genotypes a 77% lower risk for hypertension (OR=0.23, 95% CI:0.05-1.00, p=0.050) compared to the TT/DD genotype carriers.
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