7th PANHELLENIC CONGRESS OF PHARMACOLOGY

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Book of Abstracts

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.

Editor
Ass. Prof. Triaridis S

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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 TFG-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 presense 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, 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 rational 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 PROTEOGLYCANS 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 (PASMC) and increased deposition of extracellular matrix (ECM), which contributes to pathological remodelling of pulmonary arterioles in IPAH. Proteoglycans (PGs) are components of the ECM and are present throughout the lung. PGs control cellular proliferation and differentiation, however, their expression in IPAH remains elusive. The aim of our study was to investigate gene expression of: a) the large aggregating PG versican; b) the small leucine-rich repeat PG decorin and c) the basement membrane PG perlecan, in the lungs of patients with IPAH or control transplant donors.
Lung tissue samples were obtained during lung transplantation from 12 patients with IPAH (mean age 32±10 years, seven females, five males) and nine control subjects (organ donors, age 38±14 years, five females, four males). None of the IPAH patients exhibited bone morphogenetic receptor type II (BMPR2) mutations. Lung tissue specimens were homogenized and RNA was extracted and processed to cDNA. Gene expression of versican, decorin and perlecan was investigated by RT-PCR. Human hydroxymethylbilane synthase, a ubiquitously and equally expressed gene free of pseudogenes, was used as a reference gene in all RT-PCR reactions.
We found that there is a significant reduction in gene expression of the large hydrodynamic molecule versican (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 up regulated 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-methylenedioxy-N-methylamphetamin) 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 cytostaticity, 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 ecstasy 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

CYTOGENETIC 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 the dicarboxylic acid crocetin and it has been shown to have antioxidant, antidepressant, antithrombotic and mainly anticancer properties.
Purpose: 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 Proliferation Rate Index (PRI) has been established as a valuable indicator of cytostatic effect.
Methods: Lymphocyte cultures have been prepared by adding: a) 11-12 drops of heparinized whole blood from leukemic women who have not undergone any treatment and from healthy donors, b) aqueous solutions of crocin (100ng, 1μg and 10μg), in 5ml chromosome medium at the beginning of culture life. The cultures have been incubated at 37°C for 72h and SCEs and PRI has been estimated by a modification of the fluorescence plus Giemsa procedure.
Results: Findings showed that all tested crocin solutions didn't cause remarkable 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 observed, though the SCEs of healthy cells presented slight increase. Both the reduction and the increase of SCEs were proportional to the concentrations 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 significantly the DNA damages along with being demonstrated as cytoprotective.

O5

IN SILICO VIRTUAL HIGH-THROUGHPUT SCREENING APPROACH 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 virtually, against a large protein drug target database comprising over 1,000 target-proteins. Receptor–ligand molecular docking used, is a computational tool of structure based drug design, to predict protein-ligand interaction geometries 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 inhibited 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 counteract with crocin, crocetin and safranal, are acetylcholinesterase, coagulation factor VIIa, IX and Xa, serine proteinase, trypsin, neutrophil collagenase, MMP8, thymidine kinase, beta-glucosidase, thrombin, aconitate hydratase, NADPH dehydrogenase, plasminogen activator inhibitor type 1, aldo-keto reductase family 1 member C3, calmodulin, methyltetrahydrofolate, homocysteine methyltransferase, methionine synthase, elongation factor tu, alpha-amylase, thymidylate synthetase, RNA triphosphatase, tubulin, casein kinase II, protein kinase ck2, human neutrophil gelatinase and dihydrofolate 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, folic acid deficiency, homocystinuria, malaria, Parkinson's disease, hypoxic-ischemic encephalopathy, cognitive deficits, herpes virus infection, trichomoniasis, motor neurone disease, acne vulgaris, endometriosis, parkinson disease and others.
Conclusion: Our in silico molecular docking calculations provided a molecular basis for understanding the inhibitory effect of the studied compounds 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 GREEK POPULATION

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Introduction/Aim: Inosine triphosphatase (ITPA) is a cytoplasmic pyrophosphatase which recycles ITP 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 enzyme activity, with an established phenotype – genotype correlation. The two SNPs have been associated in the past with thiopurine and methotrexate drug toxicity in patients with inflammatory bowel disease (IBD), and, more recently, with protection against haemolytic anaemia in hepatitis C – infected patients treated with pegIFN/ 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 94 A 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 general population, at least 37.5% of the Greek population displays some extent of genetically determined ITPA deficiency and approximately half of those individuals could express 30% or less of the average ITPA activity of homozygotes for the major alleles of the ITPA SNPs.
Conclusions: A significant percentage of the Greek population carries a genetically determined deficiency of the ITPA enzyme, similarly to other European or American Caucasians. Genotyping IBD or hepatitis-C patients for the two SNPs primarily associated with this deficiency may be of clinical value in predicting response to thiopurines or ribavarin, respectively.

O7

AN ASSESSMENT OF THE PERCENTAGE OF PATIENTS AT POSSIBLE 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. Another 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 origin, 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 (*ABCB1* 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 reward pathway in CNS associated genes BDNF, OPRM1 and DRD2 were invastigated. 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 ethanol 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=3.79-119.92) 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.

	OR(95% CI)	P
Age (years)	1.14(1.04-1.26)	0.006
Pack years		
10-30	1.00‡	
<10	11.49(1.56-84.52)	0.016
>30	10.88(1.63-72.5)	0.014
RT-PCR		
TT	1.00	
GT/GG	21.21(3.75-119.92)	0.001

Table: Odds ratios (OR) and 95% confidence intervals (CI) derived from multiple logistic regression analysis with dependent variable the presence of prostate cancer.

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 estimated 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 were 95.4 ± 4.2 and 96.4±4.3 %, respectively. The method detection limit was established at 0.028 μg/ml and 0.029 μg/ml for 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 were 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.

Medication	Cost restriction	Comments
Quinolones	52% generic +26% brand Total : 78%	Brand offer 26 % lower price
2 nd generation cephalosporins	50%	Brand offer 16% higher price instead of 50% initially
Piperacillin/ Tazobactam	22%	New brand offer equal brand –generic price
Omeprazole	31- 40 %	Depending on generic choice
Contrast media	5- 12 %	Depending on generic choice

Medication Cost Restriction in Aretaieio University Hospital, Athens Greece, 2011
Surgical – Obstetrics, Gynaecology, Paediatric –Radiology Departments

O12

RESTRAINT STRESS TECHNIQUES AND ULCEROGENICITY IN RATS

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Introduction: Restraint 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 used in this study. Rats were divided into four groups and subjected to: i) three-hours board restraint without prior food deprivation ii) three-hours board restraint with prior food deprivation for 12 hours iii) three-hours board restraint in a cold environment (4 °C) and iv) complete food deprivation for five days. All stomachs were removed and the numbers, as well as the total length of lesions were macroscopically counted. Furthermore, histological sections were assessed for vasocongestion, epithelial cell damage, inflammation and glandular disruption.
Results: The largest number and total length of lesions was reported in the 'restraint in a cold environment' group reaching statistically significant difference compared to the other three groups. The lowest scores were observed in the 'complete food deprivation' group and the remaining two groups showed intermediate results. Microscopic examination revealed that the 'restraint in a cold environment' group had the highest score in vasocongestion and inflammation, whereas the 'restraint without prior food deprivation' group had the highest score in epithelial cell damage. Glandular disruption was observed only in six stomachs.
Discussion: Restraining the animals in a cold environment seems to be the most effective technique of the four compared in this study. Our results are in consistent with previous findings reporting that board restraint typically produces a more intense stress response and furthermore, that restraint plus cold (4°C) achieves an optimal ulcer severity effect. Future studies focusing on histological evaluation are needed in order to establish the most useful and precise technique.

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 concerned 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 ^{0}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 pretreatment 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. Furthermore, 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 differential 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 serotonergic 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-induced inhibition 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 are 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 intra-peritoneally 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 AUTONOMIC NEUROTRANSMISSION IN THE RAT: THE EFFECT OF CARNITINE

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Introduction: Choline belongs to the B_{12} complex and choline deficiency seems to impair heart function. Furthermore, choline is necessary for the synthesis of acetylcholine that regulates heart rate. The responsible enzyme for acetylcholine degradation and consequently for the termination of cholinergic neurotransmission is acetylcholinesterase (AChE); decreased myocardial AChE activity has been observed in conditions such as myocardial infarction, insulin-induced hypoglycemia, starvation and dietary protein restriction and heart failure. Among others, in the management of heart diseases, carnitine (structurally relevant to choline) has been used as an adjunct, while carnitine deficiency has been reported under states of choline deficiency.
Aim: The aim of the present study was to identify the effects of dietary choline deprivation on the myocardial acetylcholinesterase (AChE) activity in adult rats and the possible modifications after carnitine administration.
Materials and Methods: Wistar Albino rats (n=25), about 3 months old, were randomly divided into four groups: a) control (CA) (n= 6), b) rats fed with standard diet and carnitine (CARN) (n= 6), c) rats fed with choline deficient diet (CDD) (n= 7) and d) rats fed with choline deficient diet and carnitine (CDD+CARN) (n= 6). Dietary choline deprivation was induced through the administration of choline deficient diet, while carnitine supplementation was performed through drinking water (0.15% w/v). After four weeks of treatment, rats were sacrificed and myocardial AChE activity was determined spectrophotometrically to the homogenate.
Results: Rat myocardium AChE activity was significantly reduced in the CDD+CARN group compared to the CA and CDD group ($p<0.05$) as well as to the CARN group ($p<0.01$) whereas there were no significant alterations when exposed to each of the studied parameters compared to the control.
Conclusions: Choline deficiency and L-carnitine administration, separately, reduce AChE activity, but in a non-significant extent compared to the control group. In contrast, AChE activity is significantly reduced when choline deficient setting and L-carnitine administration co-exist, which implies a synergistic effect between them. Although the underlying mechanism requires further clarification, it is possible that cellular metabolic imbalances due to disturbed methyl- and acetyl-groups availability or/and cellular membrane instability may disrupt enzyme's functional potential and result to heart autonomic remodeling.

O17

INFLUENCE OF CHOLINE DEFICIENT DIET ON THE EXPRESSION OF GLUT-4 IN THE LIVER OF STZ-INDUCED DIABETIC RATS

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Introduction: Glucose Transporter-4 (GLUT-4) is an insulin-regulated glucose transporter found 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 reserves held inside cells. In the absence of insulin or muscle contraction, GLUT-4 is stored in vesicles within the cell. Defects in GLUT-4 activity have been implicated in some forms of insulin resistance and diabetes. Choline is one of the B vitamin cofactors 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 tissue, is found to be expressed even in the liver of choline deprived rats due to the high grade of fatty infiltration.
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. The diabetes was induced by an intraperitoneal injection of streptozotocin 50mg/kg BW at the beginning of the experiment. GLUT-4 mRNA was estimated by RT-PCR.
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.

POSTERS

P1

PHARMACOGENOMIC ANALYSIS OF THE ATHEROPROTECTIVE 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 homeostasis in the plasma. Furthermore, it promotes the synthesis of HDL particles that contain apoE (HDL-apoE), and are thought to contribute in 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 $\leq$0.05 false discovery rate thresholds. A total of 198 genes emerged as significantly changed between HDL-apoE3 and PBS treatments.
Results: The recombinant HDL-apoE3 particles appear to induce a number of statistically significant changes in a broad range of different molecular mechanisms directly implicated in endothelial cell function and potentially in atherosclerosis. These mechanisms include cell migration (↑COL4A1, ↑VAV3, ↑FLT1), cell proliferation (↓DKK1, ↑NR4A1, ↑HES1), cell death (↓CDK1, ↑ID1), inflammatory response (↓TGFB2, ↑PTGS2, ↓IL8), lipid metabolism (↑LIPG, ↑FABP4, ↑PPAP2B), cell cycle (↑EGR1, ↓NUF2), and signal transduction (↑PIK3CG, ↓STAT1, ↑DLL4).
Discussion: The preliminary evidence emerging from this analysis suggests the involvement of recombinant HDL-apoE3 particles in molecular pathways related to inflammation, angiogenesis and lipid metabolism. Further analysis is underway to fully define the effect of HDL-apoE3 in endothelial cell function.

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), c-Src kinase and selective G protein subunits, leading to STAT5A/B phosphorylation [4,5]. We were thus wondered whether δ-opioid receptor present in Neuro2A 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: Neuro-2A mouse and SH-SY5Y human neuroblastoma cells were cultured as described previously [6]
Differentiation and neurite outgrowth assays: Neuro-2A expressing the δ-opioid receptor were transiently transfected with the cDNA encoding the mutated His-STAT5B(Y699F) and treated with various opioid ligands or pertussis toxin [7,8]. The cells displaying neurite outgrowth were those with cellular projections of length two times greater than the cell diameter assessed 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 to 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 synaprophysin 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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P3

INTERACTIONS OF TWO MEMBERS OF THE B/R4 SUBFAMILY OF THE REGULATORS OF G PROTEIN SIGNALING WITH KAPPA AND DELTA OPIOID RECEPTORS DIFFERENTIALLY MODULATE THEIR SIGNALING

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Introduction: Regulators of G protein Signaling (RGS) comprise a large multifunctional protein family that accelerate GTP hydrolysis of Gα subunits, thus modulating G protein coupled receptor (GPCR) signaling. RGS proteins also serve as "platforms" where protein complexes can be formed [1,2]. We have previously demonstrated that RGS4 directly interacts with mu (μ-OR) and delta (δ-OR) opioid receptors to regulate their signaling [3,4]. To deduce whether selectivity in coupling between members of RGS proteins and opioid receptors exist we tested the ability of other members of B/R4-RGS family to interact with kappa (κ-OR) and δ-OR.
Methodology: Cell cultures and transient transfections: 293F or HEK293 cells stably expressing the human myc-κ-OR, or the flag-δ-OR respectively were grown and transiently transfected with either pCDNA3 or the cDNAs of HA-RGS4, or HA-RGS2 as described by [3-5].
Protein-protein interactions were detected using co-immunoprecipitation assays, and in vitro pull down assays using GST-fusion peptides encompassing intracellular portions of ORs. Subsequent experiments of cAMP accumulation measurements, detection of MAPK phosphorylation and flow cytometric analysis of cell surface receptors were performed as described previously [3-7].
Results-Discussion: In order to examine whether RGS2 interacts with opioid receptors pull down experiments were performed using HA-RGS2 and the GST fusion peptides encompassing the δ third intracellular loop (δ-i3L) and the carboxyl termini of μ (μ-CT), δ (δ-CT) and κ (κ-CT) opioid receptors. We found that RGS2 interacts with δ-i3L, δ-CT and κ-CT but not with μ-CT. Co-immunoprecipitation experiments using cell lysates co-expressing κ-OR or 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-opioid 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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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 (CRF_1). CRF_1 consists of seven plasma membrane-spanning segments (TM1-TM7), which have been recently shown by our group to form a water-accessible crevice, the binding-site crevice, which extends from the extracellular surface of the receptor into the plane of the membrane. Among the TM residues of CRF_1 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 TM3 residues are located on the surface of the binding-site crevice of CRF_1 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_1 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 receptor and are therefore potential candidates for ligand interaction. To determine the TM3 residues that are located on the surface of the binding-site crevice of CRF_1, we applied the cysteine-substituted accessibility method (SCAM), using as background the ΔCys mutant of CRF_1, and starting from the extracellular portion of TM3. The ΔCys mutant has near normal functional properties and it is relatively insensitive to the positively charged sulfhydryl-specific reagent, MTSEA. We mutated ten TM3 residues of CRF_1 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_1. The pattern of accessibility is consistent with an alpha-helical conformation for this segment of CRF_1.

P5

PHARMACOLOGICAL APPROACHES OF EXCITOTOXIC NEURODEGENERATION

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Excitotoxicity is one of the main culprits leading to neuronal cell death in many neurodegenerative conditions such as Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD), but also in epilepsy, glaucoma, traumatic brain injury (TBI), stroke or even HIV induced dementia. The term "excitotoxicity" refers to the phenomenon of neural cell death resulting from overstimulation of receptors by their respective ligands, the excitatory neurotransmitters. Glutamate receptors, that amongst other functions are responsible for the uptake of calcium ions in the cell, feature in the initiation of cytotoxic events that ultimately will lead to cell death by one of the established cell death processes namely, apoptosis, necrosis and autophagy. These events can be mediated by 1) increased intracellular calcium concentration promoting mitochondrial cytochrome C release, via depolarization of the mitochondrial membrane, initiating the caspase depended apoptotic cascade, 2) activation of calpains, a class of proteolytic enzymes, by increased calcium concentration, initiating thus the necrotic pathway, 3) activation of neuronal nitric oxide synthase (nNOS) and production of NO 4) formation of reactive oxygen species (ROS) and 5) activation of poly-ADP-ribose polymerase (PARP), which exhausts NAD^+ and ATP cellular reserves and promotes calpain mediated necrosis of the cell. Possible therapeutic interventions include pharmacological targeting for the aforementioned pathways. Agents like diazoxide, acting on an ATP sensitive K^+ channel on the mitochondrial membrane, can attenuate the intramitochondrial calcium concentration rise, attenuating also the opening of the permeability transition pore (PTP). Another approach is the direct inhibition of PTP opening by cyclosporine A or the stabilization of the mitochondrial membrane potential with triphenyphosphonium (TPP) analogues. Lastly, modified antioxidants can be used for ROS neutralization and PARP inhibitors such as 3,4-dihydro-5-[4-(1-piperidinyl) butoxy]-1(2H)-isoquinolinone (DPQ) are employed to attenuate exhaustion of cellular energy reserves.

P6

GLUTAMATE EXCITOTOXICITY UPREGULATES GRP75 AND GRP78 GENE EXPRESSION IN PC12 CELLS

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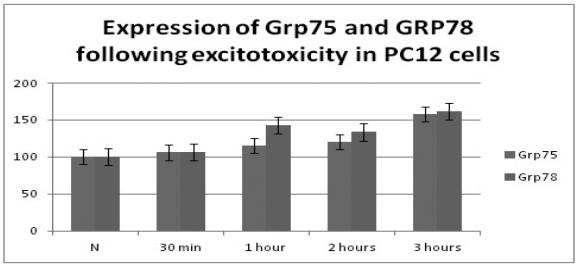
Introduction: Excitotoxicity is a phenomenon implicated in many neurodegenerative disorders leading to neuronal cell loss. Increased extracellular glutamate concentration causes an increase in calcium influx through activation of glutamate receptors. This induces intracellular stress leading to the transcription of a diverse array of proteins, that mediate cell survival. A class of proteins that are differentially expressed during cell stress, is the GRP family of proteins (glucose-regulated proteins), which exhibit chaperone activity. Rat pheochromocytoma PC12 cells serve as an appropriate experimental model cell line for studying the intracellular molecular mechanisms in neural cells. In this study we investigated the expression of Grp78, an ER resident chaperone, and Grp75 located in the internal mitochondrial membrane. In PC12 cells both proteins' expression showed a gradual upregulation in response to glutamate exposure, over time, compared to control.

Methods: The model of excitotoxicity employed, utilized exposure of PC12 cells to 10 μM glutamate in low glucose complete DMEM supplemented with 15% horse serum, 2.5% fetal bovine serum and antibiotics, at 37° C with 5% CO_2 in a humidified incubator. The same conditions were replicated for control cells, minus the glutamate addition. Cells were collected at 30 min, 1 hour, 2 hours and 3 hours after the addition of glutamate and washed three times in PBS. Cells were lysed and the protein fraction of the extract was subjected to Western Blotting.

Results: Our data indicate that Grp78 and Grp75 expression is upregulated in this experimental model of excitotoxicity employing PC12 cells. (figure).

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



P7

D_2-DOPAMINERGIC RECEPTOR LINKED PATHWAYS HOLD CRITICAL ROLE IN CYP REGULATION

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Introduction/Aim: It is well established that various hormonal and monoaminergic systems hold determinant roles in the regulation of several hepatic drug-metabolizing CYPs. Growth hormone (GH), prolactin (PRL) and insulin are involved in CYP regulation and their release is under dopaminergic control. This study has focused on the role of D_2-dopaminergic systems in the regulation of the major drug-metabolizing CYPs, the CYP3A, CYP2C and CYP2D in the liver of male rats.

Results/Discussion: The data showed that blockade of D_2-dopaminergic receptors with sulpiride (SULP) markedly down-regulated CYP3A1/2, CYP2C11 and CYP2D1 expression in the rat liver. This suppressive effect appears to be mediated by the insulin/PI3K/Akt/FOXO1 signaling pathway. Furthermore, inactivation of the GH/STAT5b signaling pathway seems also to play a role in the SULP-mediated down-regulating effect on these CYPs. SULP suppressed plasma GH levels, followed by reduced activation of STAT5b, the major GH pulse-activated transcription factor which has an up-regulating effect on various CYPs in hepatic tissue. PRL, which possesses a down-regulating control on several CYPs, was increased by SULP, and may thus also contribute in the SULP-mediated suppression of the CYPs. Finally, it appears that the SULP-induced inactivation of the cAMP/PKA/CREB signaling pathway, which is a critical regulator of PXR and HNF1α, as well as inactivation of JNK, contribute in the SULP-induced down-regulation of the above mentioned CYPs. These mechanisms could therefore also contribute to the down-regulation.

Conclusion: Taken together the present data provide evidence that drugs acting as D_2-antagonists, could interfere with several major signaling pathways involved in the regulation of CYP3A, CYP2C and CYP2D, critical enzymes in drug-metabolism, thus affecting the effectiveness of the majority of prescribed drugs, as well as the toxicity and carcinogenic potency of a plethora of toxicants and carcinogens.

P8

PLOGLITAZONE DOES NOT AFFECT BONE MINERAL DENSITY IN STREP-TOZOCIN-INDUCED DIABETIC RATS

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Background: Our study aims to investigate the effect of pioglitazone 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/cm^2) 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.

P9

AMELIORATION OF ANTIGEN INDUCED ARTHRITIS BY BEVACIZUMAB

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Introduction: Understanding the role of vascular endothelial growth factor (VEGF) in the pathogenesis of reumatoid arthritis (RA) suggests new horizons in the treatment of RA.

Aim: To investigate the therapeutic effects of intra-articular (ia) injection of bevacizumab, a humanized anti-VEGF monoclonal antibody, in an animal model of RA in vivo. We used rabbits with antigen-induced arthritis (AIA).

Methods: Pre-immunized female New Zealand white rabbits weighing 4-5 Kg were used in the study. 24h after arthritis induction using ovalbumin, the animals (N=15) were anesthetized and randomized into three groups (N=5 in each group). One group served as the disease control and was subjected to two ia injections of 0.05 mL of 0.9% NaCl on days 1, 14. Second and third treatment group were also subjected to the same dosing regimen using ia injections of 1.25 mg/0.05 mL and 2.5 mg/0.1 mL of bevacizumab respectively. All animals were sedated and sacrificed 28 days after arthritis onset.

To evaluate the grade of arthritis/inflammation, we measured the joint swelling, defined as increase in extended knee diameter from normal, using caliper and observed the grade of pannus formation as follows: 0, no involvement; 1, mild; 2, moderate; and 3, severe. Statistical analysis was performed in SPSS-19. $p < 0.05$ was considered statistically significant.

Results: Treatment with bevacizumab in both groups significantly decreased pannus formation ($p<0.05$, t-Student) and reduced joint swelling in rabbits with AIA ($p<0.05$, ANOVA) compared to control group. The observed differences between the two treatment groups were not statistically significant.

Discussion: VEGF in RA provokes angiogenesis and stimulates vascular permeability supplying oxygen and nutrients in the inflamed synovium, induces chemoattraction of peripheral leukocytes, upregulates cytokines secretion maintaining and aggravating chronic inflammation and leads to synovial hyperplasia preventing synoviocytes from undergoing apoptosis. Therefore, targeting VEGF is thought to be a promising therapeutic approach in controlling RA. Specifically blockade of articular VEGF by bevacizumab ameliorated AIA.

Conclusion: Howerver further research is necessary in order to prove bevacizumab efficacy and establish its usefulness in the treatment of RA.

P10

ACUTE EXPOSURE TO ARTIFICIAL SUNLIGHT INDUCES THE ACCUMULATION OF EOSINOPHILS IN RABBIT CONJUNCTIVAL EPITHELIUM, IN VIVO

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Introduction/Aim: Acute exposure of ocular and periocular tissues to natural or artificial light can induce inflammatory responses such as photokeratitis, anterior uveitis and conjunctivitis, attributed mainly to the UVB range of the spectrum. In this study, we have used a model of acute exposure of rabbit eyes to artificial sunlight, to study possible alterations in the architecture of corneal and conjunctival tissue as well as in the expression of selected genes related to the inflammatory response. We have also examined the effect of topical application of rupatadine, a dual H1 histamine receptor/ platelet activating factor receptor (PAFR) antagonist in the same system.
Methods: New Zealand albino rabbits were immobilized opposite a 300W 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 Jm^{-1} 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 or RNA extraction. The gene expression of tumor necrosis factor (TNF), interleukin 6 (IL-6) and PAFR was monitored with conventional RT-PCR. Rupatadine fumarate, dissolved in DMSO, was applied topically in concentrations similar to those routinely used in ocular preparations of other antihistamines, one hour before, immediately after, and one hour following exposure.
Results: No specific alterations were detected, using standard eosin-hematoxylin 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 the 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 ANTITHROMBOTIC 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, atherogenesis and other processes induced by endothelial injury.
Coumarins comprise a large class of phenolic substances occured 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 carraggenin induced rat paw edema model, have been presented from our research group. Many coumarin compounds are recognized as lipoxygenase 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, α1-antithrypsin (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_4 and AAT. Values of all other indices tended to decease 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 carragennin 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 schiatic nerve injury using the method of nerve crush in rats. The injury is achieved by crushing with forceps the schiatic 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 schiatic nerve injury in adult rats. The study was included the following: a) 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, alpha1-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 ARISTOTLE'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.
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 show that 47% of the students consume energy drinks and 30.7% declare 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 of public 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
PHARMACEUTICAL 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 sclerosus, 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 aetiology. 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, antipruritic agents. Acitretin, 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 thiophene substituted oxazole containing α-alkoxyphenylpropanoic 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,myocardial infarction 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 an 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 pandemic. 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 mh/kg b.i.d, and specifically the determination of the conversion rate of oseltamivir

phosphate to its principle metabolite oseltamivir carboxylate.
Methods: We studied pharmakokinetics of oseltamivir prophylaxis at 1.0 mg/kg bi.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 diarrheas. 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.

P18
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. Mutiple 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 R^2 was 0.29 and 0.55, respectively. In $VAS_{stillness}$, model variation's F-test was 15.50, with CRP having a coefficient 0.003827 and t-test 2.15, resulting in p=0.035 and IL-6 having a coefficient 0.002918 and t-test 4.28, resulting in p<0.0001. In $VAS_{movement}$, model variation's F-test was 45.25, with CRP having a coefficient 0.001426 and t-test 0.90, resulting in p>0.05 and IL-6 having a coefficient 0.005324 and t-test 8.78, resulting in p<0.0001.
Conclusion: In our sample size, pain scores were more dependent from Il-6, than CRP, especially in patient's movement or cough. This suggests that Il-6 is a more accurate predictor of inflammatory postoperative pain, than CRP.

P19
NECK PAIN'S ASSOCIATIONS TO STRESS BIOMARKERS
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Abstract: Pain has 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 lends credence 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

P20
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 10.12868±19.53721 in benign biopsies, whereas the respective levels of SV1 were 1.415537±2.032818 and 0.736±1.012704. 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

(SNPs) in the CYP1A2 gene have 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 I 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+1U+1X)/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 female volunteers in accordance with other Caucasian populations. The A/A genotype might confer to enhanced enzyme inducibility in conditions of exposure to CYP1A2-inducing agents, thus resulting to higher rate of inactivation of CYP1A2 substrates, such as prescribed drugs. In particular, the combination of phenotypic activity and genotypic analysis of CYP1A2 in females may provide useful information regarding the interpretation of CYP1A2 metabolic activity in diverse endogenous substrate (estrogen) fluctuations such as during the menstrual cycle, menopause or the use of drugs like oral contraceptives or hormonal replacement therapy.

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PROPOFOL BLOOD LEVELS AND THE CYP2B6 516G>T GENE POLYMORPHISM IN GREEK WOMEN

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Introduction/ Aim: Propofol is a widely used intravenous anaesthetic which displays highly variable plasma levels in different patients. It is primarily metabolized by CYP2B6, a relatively little characterized member of the cytochrome P450 family of enzymes, that has turned out to be a major catalyst in the metabolism of xenobiotics of toxicological and clinical importance. A number of polymorphisms have been recently described in the CYP2B6 gene, of which CYP2B6 516G>T is a specific marker for the *6 allele, associated with decreased expression and enzyme activity. In this study we have genotyped for CYP2B6 516G>T a small number of gynaecologic patients which received propofol for induction of anaesthesia, and compared the distribution of that polymorphism with propofol blood levels.
Methods: Twenty five premenopausal women undergoing in vitro fertilization participated in this study. Propofol was administered intravenously, for introduction to general anaesthesia, at a single bolus dose of 2.5 mg. Venous blood was removed 5 min following administration and maintained at -70ºC. Propofol levels were determined following liquid-liquid extraction and using GC/MS analysis with electron impact ionization. CYP2B6 516G>T genotyping was accomplished through an RFLP method, using DNA isolated from the same blood samples used for propofol determination.
Results: Propofol levels varied widely (Mean = 1931 ng/mL, SD = 4353 ng/mL). The CYP2B6 516G>T minor allele frequency was within the range reported for other Caucasian populations (T = 0.24). While all high propofol levels were detected in blood removed from women carriers of the T allele, the association was not statistically significant .
Conclusion: The CYP2B6 516G>T gene polymorphism displays, in our sample, a distribution similar to the one previously reported for Caucasians. No statistically significant association was detected between CYP2B6 516G>T genotypes and the distribution of propofol blood levels in our study, an effect which may be attributed to the small sample size.

P23

ASSOCIATION OF COMMON POLYMORPHISMS OF THE ENDOTHELIAL LIPASE (LIPG), THE CHOLESTERYL ESTER TRANSFER PROTEIN (CETP) AND THE LIPOPROTEIN LIPASE (LPL) GENES WITH PLASMA LIPIDS, IN DYSLIPIDAEMIC AND NORMOLIPIDAEMIC GREEKS

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Introduction-Aim: Endothelial lipase (EL) is a member of the lipoprotein lipase family of enzymes, considered to be one of the major determinants of plasma HDL cholesterol (HDL-C) levels. Cholesteryl ester transfer protein (CETP) catalyzes the exchange of cholesterol esters between HDL and very low density lipoprotein (VLDL). Together, these enzymes participate in HDL remodeling, and thus affect reverse cholesterol transport. A decrease in the activity of either enzyme leads to higher plasma HDL-C levels, a fact of obvious importance for the treatment of dyslipidaemias and the prevention/ treatment of cardiovascular disease. Lipoprotein lipase (LPL) is another enzyme that may indirectly affect CETP function as it hydrolyzes triglycerides from VLDL. All three enzymes are encoded by genes with well characterized, common polymorphisms. In this study we examine the effect of three such polymorphisms, LIPG C584T, CETP Taq1B and LPL S447X, on lipidaemic parameters, in a group of normolidaemic and dyslipidaemic individuals from northern Greece.
Methods: All patients were ethnic Greeks, diagnosed with dyslipidaemia in AHEPA University Hospital, Thessaloniki, Greece. The control group was sex- and age-matched and of the same ethnic origin. Lipidaemic parameters [total plasma cholesterol, triglycerides, HDL-C, low density lipoprotein cholesterol (LDL-C)] were determined before initiation of treatment with antidyslipidaemic drugs, through routine methods in the same hospital. Genotyping of the three polymorphisms was accomplished with previously described RFLP methods.
Results-Conclusion: Allele and genotype frequencies were similar to those published in the past for Greek (CETP Taq1B, LPL S447X) and other populations (LIPG C584T).
We have detected a possible interaction between the LIPG and CETP polymorphisms in determining HDL-C levels. A marginal association between the LPL polymorphism and body-mass index (BMI) was found in the control group.

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PREVALENCE OF POLYMORPHISMS: A→G (RS 4420638), C→T (RS 7412) AND T→C (RS 429358), A→G (RS 157581), G→C (RS 3745833) OF APOC1, APOE, TOMM40 AND GALP GENES, RESPECTIVELY, ASSOCIATED WITH ALZHEIMER'S DISEASE, IN A SAMPLE OF GREEK POPULATION

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Introduction: Alzheimer's Disease (AD) is the most common cause of senile dementia. Although it is fostered by genetic predisposition, it is a multifactorial disease, also affected by various environmental factors. A large number of AD related genes has been discovered and studied, having more or less participation in the disease's progression. In the present study, after briefly describing AD, we will discuss four genes associated with it: APOE, APOC1, TOMM40 and GALP. Firstly we will investigate the mechanisms with which the genes affect AD and finally their prevalence on a Greek population sample.
Materials and Methods: The study's population is 296 Greek individuals, 163 of whom are females and the rest 133 males. With DNA extraction method, DNA was isolated from the epithelial cells of volunteers which were collected using sterile cotton swab. The detection and identification of the genes was done with the PCR (polymerase chain reaction) technique.
Results: As for APOC1 (polymorphism A→G), 222 of the participants bared the wild type of the gene, A:A, 71 were heterozygous and 3 bared the G:G alleles. Homozygous for the APOE gene (polymorphism C→T) wild type, C:C, were 266 individuals, while 28 bared the C:T alleles and 2 the T:T alleles. As for the T→C polymorphism of the same gene, 251 individuals were found with the T:T genotype, which is the wild type, 44 with C:T and none with the C:C alleles. On the TOMM40 gene (polymorphism A→G) 201 bared the A:A genotype, (wild type), 90 bared the A:G genotype, while 5 participants bared the G:G genotype. As for GALP, finally,

(polymorphism G→C) 123 individuals were found with the wild type G:G, 132 with the G:C alleles and 41 with the C:C alleles.
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%CL: 1.12-2.08; p=0.008). Participants with genotype T/C-A/A-A/A were more susceptible to the disease (OR=2.13; 95%CL: 1.02-4.47; p=0.045). Similarly, participants with T/T-A/A-G/A were more likely to develop the disease (OR=3.20; 95% CI: 1.02-10.05; p=0.046). 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/DI 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/DI 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/DI 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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