# 9th PANHELLENIC CONGRESS OF PHARMACOLOGY

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Book of Abstracts

Dear attendees of the 9th Biannual Congress of the Hellenic Society for Basic and Clinical Pharmacology, the Organizing Committee welcomes you to Thessaloniki.

Our Society's Biannual Meetings and Congresses serve as an opportunity for Greek pharmacologists and other interested scientists and professionals to come together and exchange views and information in various aspects of Pharmacology – basic and clinical. Even though the Organizing Committee of this year's Congress has worked under financial and time constraints, we have managed to secure the participation of a small number of experts, Greek and foreign, in fields such as drug development, pharmacoepidemiology, regulatory pharmacology, pharmacogenomics and therapeutic drug monitoring, and target validation in the pharmacology of the cardiovascular system. As always, this year's Congress will provide the opportunity to young researchers to present their ideas and experimental results and receive feedback from more experienced scientists. Finally, for the first time a Pharmacology Olympiad will take place, in which students will have the opportunity to compete for best knowledge and understanding of pharmacological concepts.
We hope that you will all enjoy the 9th Biannual Congress of the Hellenic Society for Basic and Clinical Pharmacology.

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Professor
Head of the Organizing Committee

HIPPOKRATIA

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# ORAL PRESENTATIONS

OP1

## THE NITRIC OXIDE DONOR SODIUM NITROPRUSSIDE ATTENUATES RECOGNITION MEMORY DEFICITS AND SOCIAL WITHDRAWAL PRODUCED BY THE NMDA RECEPTOR ANTAGONIST KETAMINE AND INDUCES ANXIOLYTIK-LIKE BEHAVIOUR IN RATS

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Introduction: Experimental evidence indicates that the non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist ketamine impairs cognition and can mimic certain aspects of positive and negative symptoms of schizophrenia in rodents. Nitric oxide (NO) is considered as an intracellular messenger in the brain and its abnormalities have been linked to schizophrenia.
Aims: The present study was designed to investigate the ability of the NO donor sodium nitroprusside (SNP) to counteract schizophrenia-like behavioural deficits produced by ketamine in rats.
Methods: The ability of SNP to reverse ketamine-induced memory deficits and social withdrawal were assessed using the novel object recognition task (NORT) and the social interaction test respectively. Further, since anxiety disorders are noted to occur commonly in schizophrenics the effects of SNP on anxiety-like behaviour were examined using the light/dark test. Locomotor activity was also assessed as an independent measure of the potential motoric effects of this NO donor.
Results: SNP (0.3 and 1 mg/kg) reversed ketamine (3 mg/kg)-induced short-term recognition memory deficits. SNP (1 mg/kg) counteracted the ketamine (8 mg/kg)-induced social isolation in the social interaction test. The anxiolytic-like effects in the light/dark test of SNP (1 mg/kg) cannot be attributed to changes in locomotor activity.
Conclusions: Our findings illustrate a functional interaction between the nitrergic and glutamatergic system that may be of relevance for schizophrenia-like behavioural deficits. The data also suggest a role of NO in anxiety.

OP2

## IN VITRO AND IN VIVO EFFECTS OF THE CHEMOTHERAPEUTIC DOCETAXEL AND THE BCR/ABL KINASE INHIBITOR DASATINIB IN TRIPLE- NEGATIVE BREAST CANCER: THE EXPERIMENTAL SET-UP

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Introduction: The term "triple-negative breast cancer" (TNBC) is used to identify the approximately 15% of breast cancers that lack expression of estrogen receptor (ER) and progesterone receptor (PR) and do not show amplification of the human epidermal growth factor receptor 2 (HER2) gene. TNBCs are a heterogeneous group of tumors with one common feature: a distinctly aggressive nature with higher rates of relapse and shorter overall survival in the metastatic setting compared to other subtypes of breast cancer.To date, not a single targeted therapy has been approved for the treatment of TNBC, and cytotoxic chemotherapy remains the standard treatment.
Aim: The experimental study of the effects of the chemotherapeutic docetaxel and the bcr/abl kinase inhibitor dasatinib on triple- negative breast cancer cell lines (in vitro) and onTNBC tumor xenograft mouse models (in vivo).
Methods: TNBC cell lines were cultivated and treated with various concentrations of docetaxel and dasatinib (5 nM to 100nM). Cell death and apoptosis were studied by flow cytometry. TNBC cell lines were then injected in BALB/c athymic nude mice to express the tumor in vivo. Four groups of mice were created (group A: Control, group B: DOC, group C: DAS, group D: DOC + DAS) and treated respectively with the drugs and their combination. Tumors were obtained, maintained in formaldehyde solution of 10%, embedded in paraffin and sent for further histological evaluation (hematoxylin- eosin staining and immune-histochemical analysis) to assess the tumor growth inhibition.
Results: Preliminary results on the cytotoxic effects of docetaxel seem to be statistically important, with little to no effect on apoptosis. Current work concerning the effect of dasatinib in vitro and vivo is in progress.
Conclusion: TNBC is a difficult to treat oncologic condition, even in the experimental setting. Promising results concerning the addition of targeted therapies to the conventional cytotoxic ones (docetaxel) have been shown, awaiting further evaluation.

OP3

## HYDROGEN SULFIDE DONORS REDUCE CELL ADHESION AND EXPRESSION OF INFLAMMATORY SIGNALING MEDIATORS IN ENDOTHELIAL CELLS

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Background & Objectives: A key event in atherogenesis is monocyte cell adhesion to endothelium. This process is mediated by inflammatory cytokines such as Monocyte chemmoattractant protein-1 (MCP-1) and expression of adhesion molecules such as Inter-cellular adhesion molecule 1 (ICAM-1). Hydrogen sulfide ($H_2S$) appears to confer cytoprotection via multiple mechanisms including anti-inflammatory effects. The present study aimed to investigate the possible vascular anti-atherosclerotic effect of two $H_2S$ donors, GYY4137 and thioglycine in an *in vitro* model.
Methods: EAhy926 endothelial cells were grown to confluence in 12-well plates. Cells were serum-starved and then incubated with several concentrations of GYY4137 and thioglycine for 1h and then with TNF-α (10ng/ml) for 6h. Medium was then removed, THP-1 monocytes were seeded on TNF-α activated EAhy926 monolayers and co-incubated for 1h at 37°C. Adhesion was counted on randomly selected magnification microscopic fields/well. Total RNA was extracted from EAhy926 cells (treated with thioglycine and GYY4137 with several concentrations for 1h and alone or in parallel with TNF-α (10ng/ml) for 2h) and reverse-transcribed into cDNA. qPCR experiments for MCP-1 and ICAM-1 mRNA levels were performed and expression levels were evaluated for each gene by comparing samples treated with $H_2S$ donors with negative control treatment.
Results: Control-confluent EAhy926 showed minimal binding for THP-1 cells, while the adhesion of THP-1 was significantly increased to TNF-α-stimulated EAhy926. Pre-treatment of Eahy926 with increasing GYY4137 (300μM & 500μM) or thioglycine (100μM, 300μM & 500μM) concentrations for 1h before TNF-α activation caused a concentration dependent inhibition of THP-1 adhesion up to 69.3% ± 2.2 % and 48.3% ± 2.8 %, respectively. MCP-1 and ICAM-1 mRNA levels were significantly reduced in EAhy926 cells co-incubated with TNF-α and GYY4137 or thioglycine in a dose-dependent manner, compared to TNF-α alone. When applied alone, both $H_2S$ donors were more effective in suppressing gene expression, compared to co-incubation with TNF-α.
Conclusion: $H_2S$ interferes with pro-inflammatory and pro-atherogenic processes by inhibiting monocyte cell adhesion and attenuating expression of MCP-1 and ICAM-1 in inflamed endothelial cells.

OP4

## THIOLS ACT AS PREVENTIVE AGENTS FOR HEME-INDUCED CELLULAR DAMAGE IN K562 CELLS

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Heme is inherently dangerous upon release from its intracellular sites. As a highly lipophilic molecule, heme readily enters adjacent cells provoking massive oxidative destruction. Multiple disorders are accompanied by hemolysis (like sepsis and hemorrhagic strokes). Despite this, there is no effective therapy at the clinical setting to manage free heme toxicity. In preliminary studies involved combination of hemin and N-acetyl cysteine (NAC), a rather unexpected observation emerged: NAC resulted in markedly attenuated intracellular heme content. The central aim was to uncover the mechanism via which thiols prevent hemin-induced cell damage (HICD). K562 pro-erythroid cells were used as a cell model. qPCR and Western blot analysis were complemented by the pyridine chemochromagen assay (measurement of intact heme content), the Ferrozine assay (measurement of free iron) and dichlorofluorescin (a redox-sensitive probe) to address more specific experimental issues. The results are summarised below. a) HICD was characterized by sequential cell growth arrest and death, b) The intracellular accumulation of intact heme increased whereas an incremental up-regulation of heme oxygenase-1 (Ho-1), the heme catabolising enzyme was recorded. c) NAC maintained safe intracellular heme levels; this undoubtly contributed to abrogation of HICD. d) Both cysteine and GSH extrapolated the effects of NAC on HICD. e) Heme and thiols interacted leading to structural instability of hemin and release of the iron atom. f) The cells protected from HICD by thiols were accompanied by a basal redox state. g) The ROS scavenging property is not neither adequate nor a prerequisite for abrogation of HICD. h) Prevention of HICD by thiols was accompanied by a moderate induction of Ho-1. It can be concluded that a lower induction of cells defence mechanisms was sufficient to cope with the attenuated heme pools in presence of thiols. This study set the fertile ground for developing effective preventive therapeutics for severe hemolytic disorders.

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OP5

**LEVOSIMENDAN INDUCED CARDIOPROTECTION AGAINST ACUTE DOXORUBICIN CARDIOTOXICITY THROUGH ACTIVATION OF ANTIOXIDANT, ANTIAPOPTOTIC MECHANISMS AND METABOLIC RESTORATION**

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Introduction: Adriamycin (Doxorubicin-DXR) is a widely applicable antineoplastic drug effective against various cancers types. However its antitumor activity is accompanied by acute and chronic cardiotoxicity. Levosimendan (LEVO), is a clinically used drug with inotropic and vasodilator activity, indicated for the treatment of irreversible heart failure. The main pharmacological properties of the compound are increased cardiac contractility and cardioprotection. As we have previously shown LEVO does not affect the antitumor activity of DXR, as proven *in vitro* on PC3 cell line and reduces morphological alterations of the myocardium attributed to acute doxorubicin cardiotoxicity in an *in vivo* rat model.

Purpose: We sought to investigate the underlying protective mechanism of levosimendan against acute doxorubicin-induced cardiotoxicity, focusing on antioxidant, antiapoptotic and metabolic pathways. Methods: Male rats were randomized into the following groups: 1.Control (N/S) 2.DXR (DXR 20mg/kg) 3.DXR+LEVOA (DXR 20mg kg+LEVO 12mg/kg) 4.DXR+LEVOB (DXR 20mg/kg+LEVO 24mg/kg). On the third day after the administrations echocardiography was performed and myocardial samples were obtained for determination of nitro-oxidative stress biomarkers levels (MDA, PCs, Nitrotyrosine), mRNA levels of MMP2, TGF-b1 and protein expression of iNOS, phospho-Akt/Akt, phospho-ERKs/ERKs, MnSOD, IL-6 and Nox-4. Moreover heart tissue extracts underwent metabolomic analysis by means of $^{1}$H-NMR for evaluation of changes on the metabolome among groups.

Results: Levosimendan reduced oxidative stress levels and IL-6 in a dose-dependent manner (DXR+LEVOB vs DXR, $p<0.05$). Moreover, Levosimendan decreased mRNA levels of MMP2 and levels of iNOS (DXR+LEVOA vs DXR $p<0.05$; DXR+LEVOB vs DXR, $p<0.01$) and NOX-4(DXR+LEVOB vs DXR, $p<0.03$), while there was an increase of MnSOD expression and phosphorylation of Akt (DXR+LEVOB vs DXR, $p<0.05$). No change was observed in the levels of TGF-b1 and in the phosphorylation of ERKs. Moreover PCA (3PCs, R2(cum)=0.52, Q2(cum)=0.29) and PLS-DA (3PCs, R2(cum)=0.49, Q2(cum)=0.34) analysis of the myocardial metabolites declared that LEVO dose-dependently restored metabolic phenotype similar to control group, reduced the concentration of beta-branched aminoacids by inhibiting protein degradation and abrogated anaerobic glycolysis resulted as a metabolic switch due to DXR-induced cardiotoxicity.

Conclusion: Levosimendan displays protective effects against acute cardiotoxicity of adriamycin by regulation oxidative stress, activation of antiapoptotic mechanisms and restoration of myocardial energy metabolism.

OP6

**HEPATOTOXIC MEDICINAL PLANTS**

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Introduction – Aim: Herbal products rise in popularity as a life style, due to cheaper costs, easy availability, patient compliance and mainly because they are regarded generally by the public, as harmless remedies for self-medication without supervision.

However, toxicity of herbal supplements is being increasingly recognized. Especially liver toxicity following consumption of herbal remedies is on the increase. Thus, there is an urgent need to understand the action of the herbal supplements on the liver.

Clinical manifestations include abdominal pain, jaundice, ascitis, hepatomegaly, raised serum transaminase levels, hepatocellular and cholestatic hepatitis. Prognosis for some herbs is good, the disease is resolved after discontinuing the intake of the herbal- readministration is followed by the prompt recurrence of hepatitis, but for other products the recovery is poor with need for intensive therapy - liver transplant and death rates of 20 to 30% being reported. The frequency and dose-dependency of the hepatic damage is unclear. For some products the picture is confused further by demonstrations of hepatoprotective properties for some components.

The present review focuses on several herbs which are reported to cause hepatotoxicity in humans and animals:

Actaea racemosa, Aloe barbadensis, Artemisia absinthium, Atractylis gummifera, Calendula officinalis, Callilepsis laureola,Camellia sinensis, Centella asiatica, Chelidonium majus, Ephedra sinica, Garcinia camboge, Glycyrrhiza glabra, Hypericum perforatum, Larea tridentate, Lycopodium serratum, Manihot esculenta, Mentha pulegium, Mitragyna speciosa, Morinda citrifolia, Piper methysticum, Polygala chinensis, Scutellaria biacalensis, Scutellaria lateriflora, Senna (Cassia acutifolia & Cassia angustifolia), Symphytum officinale, Teucrium chamaedrys, Teucrium polium, Usnea species,Valeriana officinalis, Xanthium strumarium and some chinese herbal medicines such as Jin Bu Huan ,Ma-Huang and Sho-

saiko-to, containing one or more of the above herbs.
Methods: Current literature on the hepatotoxicity of herbal drugs is reviewed.
Results – Discussion: The documented toxicity of these alternative plant remedies emphasizes the importance of pharmacovigilance and quality control in the manufacture of these products. Consumers should use only those medicinal herbs established by formal Health Regulatory Organizations as European Medicines Agency. Clinicians should always inquire about herbal products intake in cases of unexplained liver injury.

OP7

**CHANGES IN ANTIPSYCHOTIC PRESCRIBING FOR OUTPATIENTS OF AHEPA HOSPITAL DURING THE YEARS OF FINANCIAL CRISIS IN GREECE**

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Introduction: Under the current financial crisis in Greece, an effort has been made by the Greek health authorities and by Greek physicians to lower medicinal cost.
Aim: The purpose of this work was to study trends in antipsychotic prescribing and in utilization of generics in outpatients of AHEPA Hospital of Thessaloniki, during the years of the financial crisis.
Methods: Two samples of antipsychotic prescriptions corresponding to the first four months of the years 2009 and 2015 were collected from the archives of the Psychiatry Outpatient Department of the AHEPA Hospital in Thessaloniki, Greece. All proprietary names of antipsychotics and their relative ratios in the prescriptions were estimated, and the percentage of generics in prescriptions was calculated. The amount of prescribed medicines was estimated in Defined Daily Doses (DDDs) of the reference drug and its generics. The comparison of the two samples was made by using the statistical package SPSS.
Results: The total number of prescriptions increased from 21,879 DDDs in 2009 to 47,373 DDDs in 2015. Haloperidol, risperidone, olanzapine, quetiapine and perphenazine/amitryptiline were the most prescribed antipsychotics in both samples, accounting for 83% of all antipsychotics prescribed in 2009 and 79% of all antipsychotics prescribed in 2015. Generic prescribing increased dramatically from 2009 to 2015, corresponding to 24% of total antipsychotic prescriptions in 2015 and 4% of total antipsychotic prescriptions in 2009. The percentage of generics was high for olanzapine (61%), risperidone (60%) and amisulpride (60%) in 2015, while in 2009 the percentage of generics was high only for risperidone (19%).
Conclusions: The total number of prescriptions and the percentage of generics in antipsychotic prescribing were much higher in 2015 than in 2009. These results reflect an increase in the number of people seeking medical advice in the National Health System, and an increase in the efforts of Greek physicians to lower medicinal cost.

OP8

**SELF-MEDICATION PRACTICES WITH ANALGESIC DRUGS AMONG GREEK MEDICAL STUDENTS**

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Objective: Self-medication is a major reason for various adverse events related with analgesic drugs. The aim of the present study was to assess practice and perception of self medication among medical students. Materials-Methods: A questionnaire-based, cross-sectional survey, concerning economic conditions and health practices of non steroidal anti-inflammatory drugs (NSAIDS) self administration, for the previous 6-months time period, was conducted in Faculty of Medicine, School of Health Sciences, Aristotle University of Thessaloniki, from January to April 2015. A total of 700 copies of questionnaires were distributed. A total of 531 forms were completed and returned (response rate 75.8%). Results: According to the results, 414 (77.9%) students had a monthly income of less than 300 euro, while 97 (18.3%) students did not have any health insurance. Out of 531 students that responded, 310 (58.3%) were self-medicated with analgesic drugs the last 6 months. The principal symptoms for seeking self medication with NSAIDS drugs were abdominal pain 145 (46.8%) and headache as reported from 165 students (53.2%). Out of 310 self medicated students only 205 (66.1%) knew the right daily dosage of the analgesic drug they were taking, while 105 of them (33.9%) were taking wrong dosages during the treatment period and 45 (14.5%) reported adverse events related to analgesics. The main reason reported for not visiting a medical doctor, 189 students (61%), was the prior use of the same antibiotic in the past. Female gender and older age were identified as independent risk factors of self medication with NSAIDS. Conclusions: Self administration with NSAIDS drugs is common between medical students in Greece and can often lead to wrong dosages or adverse events. Better regulation on analgesic sales and education reinforced by further health care reform are recommended.

OP9

**ASSOCIATION OF *ABCB1* POLYMORPHISMS WITH HALOPERIDOL-BASED TREATMENT OF SCHIZOPHRENIA IN NATURAL SETTING: A PRELIMINARY REPORT**

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Introduction-Aim: ATP-binding cassette, subfamily B, member 1 gene (*ABCB1*) polymorphisms have been associated in the past with response to antipsychotic drug therapy and severity of illness in patients suffering from schizophrenia or schizoaffective disorders, but the extent of association is expected to vary with clinical setting. In this study we examined the association between *ABCB1* polymorphisms G2677T/A (rs2032582) and C3435T (rs1045642) and severity of illness at presentation (PANSS baseline score/ PANSS base), antipsychotic drug (haloperidol) concentration at steady state, average drug requirements (daily chlorpromazine equivalents/CPZeq) and efficacy of drug therapy.
Methods: Forty three patients suffering from schizophrenia or schizoaffective disorders participated in this study. The study was undertaken in its natural setting with drugs administered as per patients' need. Patients received haloperidol in combination with amisulpride, olanzapine, paliperidone, quetiapine, risperidone or sertindole . Genotyping was achieved with established PCR-RFLP methods. Response following one month of treatment was assessed with the PANSS scale and was defined as a ≥50% change in the total PANSS score. Haloperidol levels were determined in whole blood with an LC-MS/MS method. Associations between genotypes and PANSS base, haloperidol concentration, and CPZeq were tested

with ANCOVA using age, gender, age of onset and PANSS base or diagnosis of schizophrenia as covariates. Comparison of genotype frequencies between responders and non-responders was done with the $\chi^2$ test of independence. Multiple regression analyses were used to establish predictor variables for CPZeq and blood haloperidol levels.
Results: The *ABCB1* polymorphisms were significantly associated with haloperidol levels, with TT genotypes displaying higher blood concentrations (p = 0.008 and p = 0.012, for rs2032582 and rs1045642 respectively) but not with average daily drug requirements. Only rs2032582 was a statistically significant predictor of haloperidol concentrations however (together with PANSS base). Both haloperidol levels and CPZeq were weakly but significantly correlated with PANSS base (ρ = 0.334, p = 0.028; ρ = 0.205; p = 0.041), and with each other (ρ = 0.354, p = 0.020). No significant differences in genotype frequencies between responders and non-responders to treatment were detected. Severity of illness was not associated with either of the two polymorphisms.
Conclusion: In a natural clinical setting characterized by polypharmacy, rs2032582 was a statistically significant predictor of haloperidol concentrations but not of average daily CPZeq, suggesting that the polymorphism may affect haloperidol's relative efficacy compared to the other drugs.

OP10

**EPIGENETIC MODIFICATIONS OF *BRMS1* GENE AND PROTEIN EXPRESSION IN UTERI CERVIX IN RELATION TO HIGH-RISK HPV INFECTION AND CANCER.**

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DNA methylation is a well-characterized epigenetic mechanism with a significant role in regulating gene expression. Cervical cancer which is the fourth most common cancer in women and high-grade intraepithelial lesion are strongly related to certain types of HPV infection (HR-HPV). Breast cancer metastasis suppressor 1 (BRMS1) suppress metastasis and cell invasion without preventing primary tumor growth. Its expression is down regulated by methylation in several types of cancers and is correlated with disease progression and poor prognosis. However, its role in cervical cancer as well as in precancerous lesions has not yet been clarified.
In the present study, we compared the methylation status of *BRMS1* gene promoter in cervical smears of 64 women with no infection by HR-HPV (16, 18, other high risk strains) to 70 women with proven infection with varying CIN stage, using real-time methylation-specific PCR (real-time MSP) and a methylation-independent assay for the β-actin gene (*ACTB*) to verify DNA quality. Additionally, the expression of BRMS1 protein was studied immunohistochemicaly in paraffin embedded cervical cancer tissue and precancerous lesions.
Surprisingly, methylation of *BRMS1* promoter was detected more often in women with no HPV infection than in infected women ($p$<0.001, $x^2$ 12.502), but was not related to a specific HPV subtype (p<0.001). In HPV subgroup methylation of BRMS1 was not related to CIN stage (p=0,859, $x^2$ 0.032) and cytology (p=0,683, $x^2$ 0.167). In addition, methylation of BRMS1 gene promoter was present more often in older women (p=0.013, x2 6.236). Notably, we have detected *BRMS1* hypermethylation in Hela cervical cancer cells. Moreover, the expression of BRMS1 protein was found in normal as well as in cancerous and precancerous cervical tissues.
Our results show a correlation between *BRMS1* unmethylated gene promoter and HR-HPV infection in cervical epithelium. Aberrant BRMS1 protein expression might be related to the initiation of carcinogenetic process. A larger study is necessary in order to determine the role of BRMS1 in cervical cancer.

OP11

**DEVELOPMENT AND VALIDATION OF A REVERSED-PHASE HPLC METHOD FOR THE THERAPEUTIC DRUG MONITORING (TDM) OF LICARBAZEPINE IN PATIENTS UNDER OXCARBAZEPINE TREATMENT**

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Introduction: Oxcarbazepine is used in the treatment of focal epilepsy. Following its absorption, it is reduced to its mono-hydroxy derivative licarbazepine which is responsible for the antiepileptic action of oxcarbazepine. Oxarbazepine and licarbazepine follow linear elimination pharmacokinetics mainly through non-oxidative processes independent of the CYP system, thus, exhibiting a relatively low potential for enzyme induction or inhibition and, consequently, less drug interactions and side effects compared to other antiepileptic drugs. Nevertheless, oxcarbazepine reduces the efficacy of oral contraceptives by affecting metabolism (CYP3A) or protein binding. Moreover, licarbazepine clearance is reduced in the elderly and in individuals with renal insufficiency and is increased in pregnancy. Several HPLC methods developed for serum licarbazepine TDM exhibit disadvantages due to high cost (mass spectrometry) or low sensitivity and/or prolonged sample pretreatment (UV-based methods). The study aims at developing a simple, low cost, specific and sensitive chromatographic method for serum licarbazepine TDM in patients treated with oxcarbazepine.
Methods: Serum samples of epileptic patients (n=13) and calibration and control samples underwent protein precipitation using methanol. Chromatographic separation was observed within 9min using a $C_{18}$ reversed-phase column and mobile phase consisting of 50mM $NaH_2PO_4(H_2O)/CH_3CN$ (70/30, v/v, pH=4) delivered isocratically (0.8mL/min, 30°C). Detection was set at 210nm.
Results: The calibration curve was linear (regression coefficient=0.998) over the range 0.5-50.0μg/mL. The recovery range was 99.49-104.52% and the lowest limit of quantitation was 0.2μg/mL. No interference between licarbazepine/internal standard and more than 50 possibly co-administered medications tested (antiepileptics, antipsychotics, antidepressants, benzodiazepines, antiarrhythmics, antibiotics etc) was observed. The method developed in the present study was implemented to clinical samples and the concentration of licarbazepine was determined in plasma samples obtained from patients under oxcarbazepine treatment either as monotherapy or in polytherapy with other antiepileptic drugs. Licarbazepine serum concentrations were significantly (p=0.02) correlated with oxcarbazepine dose (Pearson correlation coefficient: 0.66).
Conclusions: A simple, fast, low cost and sensitive chromatographic method was developed for serum licarbazepine TDM in epileptic patients. The lack of interference with antiepileptics, antipsychotics and other commonly used drugs renders this method an important tool in clinical practice and contributes toward therapy optimization and minimization of toxic effects.

# POSTERS

PP1

## CHANGES IN GENERIC PRESCRIBING OF ANTIDEPRESSANTS DURING THE YEARS OF FINANCIAL CRISIS IN GREECE

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Introduction: Under the current financial crisis in Greece, an effort has been made to increase generic prescribing, in order to lower medicinal cost[1-4]. Aim: The purpose of this work was to study trends in utilization of generics in antidepressants sales in the market of Thessaloniki, during the years of the crisis.
Methods: Two samples of antidepressants registered sales corresponding to the years 2012-2013 and 2014-2015 were collected for the study. The samples corresponded only to a small amount of sales from the market of Thessaloniki. All classes of antidepressants and their relative ratios in the sales were estimated, and the percentage of generics in the sales of each medicine was calculated out of a variety of brand names in each class of antidepressants. The amount of medicines was estimated in Defined Daily Doses (DDDs) of the reference drug and its generics. The comparison of the two samples was made by using the statistical package SPSS.
Results: Generic use corresponded to 26% of total sales of antidepressants in the sample of the years 2012-2013 and 32% of total sales in the sample of the years 2014-2015. During the last two years the percentage of generics increased from 36% to 52% in sertraline sales, from 44% to 56% in venlafaxine sales and from 53% to 65% in citalopram sales.
Conclusions: Under the financial crisis in Greece, an increase in generic use was observed in antidepressant sales in the market of Thessaloniki.

References:
1. Papaioannidou P, Ntaralas A. Basic & Clinical Pharmacology & Toxicology 2014, 115(SI):70
2. Papaioannidou P, Ntaralas A. Pharmacoepidemiology and Drug Safety 2014, 23(SI):115
3. Papaioannidou P, Ntaralas A. Clinical Therapeutics 2015, 37(8S):e148
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PP2

## ATTITUDES IN THE USE OF ANTIHYPERTENSIVES IN THESSALONIKI

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Introduction: There are not many studies on utilization of drugs in Greece[1-4].
Aim: The purpose of this work was to study utilization of antihypertensives in the market of Thessaloniki.
Methods: A sample of antihypertensives registered sales corresponding to the year 2015 was collected for the study. The sample corresponded only to a small amount of sales from the market of Thessaloniki. All classes of antihypertensives and their relative ratios in the sales were estimated, and the percentage of generics in the sales of each medicine was calculated out of a variety of brand names in each class of antihypertensives. The amount of medicines was estimated in Defined Daily Doses (DDDs) of the reference drug and its generics. The statistical package SPSS was used for statistical analysis.
Results: Angiotensin II receptor antagonists, calcium channel blockers, beta blockers, ACE inhibitors and diuretics were the most prescribed antihypertensives in the study sample, corresponding respectively to 32%, 24%, 16%, 15% and 12% of antihypertensives registered sales. Generic use corresponded to 15% of total sales of antihypertensives.
Conclusions:. Angiotensin II receptor antagonists and calcium channel blockers were the most popular antihypertensives in the study sample. The use of generics was not high.

References:
1. Papaioannidou P, Ntaralas A. Basic & Clinical Pharmacology & Toxicology 2014, 115(SI):70
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PP3

## ANTIPSYCHOTIC PRESCRIBING FOR INPATIENTS OF AHEPA HOSPITAL

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Introduction: Under the current financial crisis in Greece, an effort has been made to lower medicinal cost[1-4].
Aim: The purpose of this work was to study antipsychotic prescribing and utilization of generics in patients of AHEPA Hospital in Thessaloniki.
Methods: A sample of antipsychotics prescriptions corresponding to the first six months of the year 2015 was collected from the archives of the Psychiatry Department of the AHEPA Hospital in Thessaloniki, Greece. All proprietary names of antipsychotics and their relative ratios in the prescriptions were estimated, and the percentage of generics in prescriptions was calculated. The amount of prescribed medicines was estimated in Defined Daily Doses (DDDs) of the reference drug and its generics. The statistical package SPSS was used for statistical analysis.
Results: Prescriptions of haloperidol corresponded to more than half of total prescriptions (53%). Risperidone, olanzapine and quetiapine were the other three most prescribed antipsychotics, corresponding to 33% of total prescriptions. Generic prescribing corresponded to 15% of total antipsychotic prescribing. The percentage of generics was around 50% for risperidone and olanzapine.
Conclusions: In the study sample, older and cheaper antipsychotics or generics were prescribed in most cases. These results reflect the efforts of Greek physicians to lower medicinal cost under the current financial crisis in Greece.

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1. Papaioannidou P, Ntaralas A. Basic & Clinical Pharmacology & Toxicology 2014, 115(SI):70
2. Papaioannidou P, Ntaralas A. Pharmacoepidemiology and Drug Safety 2014, 23(SI):115
3. Papaioannidou P, Ntaralas A. Clinical Therapeutics 2015, 37(8S):e148
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PP4

**ATTITUDES IN HORMONAL CONTRACEPTION IN THESSALONIKI**

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Introduction: The use of hormonal contraceptives is not popular and it is generally very low in Greece.
Aim: The purpose of this work was to study sales of hormonal contraceptives in a sample from the medicines market of Thessaloniki.
Methods: A sample of hormonal contraceptives registered sales corresponding to the years 2014-2015 was collected for the study. The sample corresponded only to a small amount of sales from the market of Central and Eastern Thessaloniki. All kinds of hormonal contraceptives in the sales were estimated, and the consumption of contraceptives was expressed in months of contraception. The statistical package SPSS was used for statistical analysis.
Results: In the study sample, sales of systematic oral contraceptives corresponded only to 3,825 months of contraception out of a total of 6,318 months of contraception (61%). The percentage of emergency contraception was very high (25%), corresponding to 1,593 months of contraception. The sales of intrauterine systems of hormonal contraception were very limited (less than 0.3% of sold boxes) but corresponded to 900 months of contraception (24%), thanks to the extended release of levonorgestrel and the long duration of contraception of these systems (5 years). The most common type of oral contraceptives was the combination of ethinylestradiol and drospirerone.
Conclusions: The sales of hormonal contraceptives in the market of Thessaloniki was low. The percentage of emergency contraception was very high, while the percentage of intrauterine hormonal contraception was very limited.

PP5

**SCAVENGER RECEPTOR CLASS B TYPE I REGULATES PLASMA APOLIPOPROTEIN E LEVELS AND DIETARY LIPID DEPOSITION TO THE LIVER**

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Introduction: Based on previous work in our laboratory, apolipoprotein A-I (apoA-I), the main protein component of HDL and lecithin:cholesterol acyl transferase enzyme (LCAT), play important role in the development of diet-induced obesity, diabetes and non-alcoholic fatty liver disease (NAFLD) in mice suggesting that the HDL metabolic pathway may play important role in these processes. Therefore, in the present study we investigated the potential role of scavenger receptor class B type I (SR-BI) in these metabolic disorders since it serves as the main receptor of HDL and it is primarily responsible for the uptake of cholesteryl esters (CE) of HDL particles by liver.
Methods: To test the effects of SR-BI in those metabolic disorders, female SR-BI-deficient (scarb1$^{-/-}$) mice and wild type (WT) C57BL/6 mice were fed western type diet for 24 weeks and afterwards biochemical and histological analysis were performed.
Results: Analysis of the apolipoprotein composition of plasma lipoproteins revealed a significant accumulation of apolipoprotein E (ApoE)-containing HDL and TG-rich lipoproteins in scarb1$^{-/-}$ mice that correlated with reduced plasma LpL activity. Biochemical and histological analysis of liver showed that scarb1$^{-/-}$ mice had decreased diet-induced hepatic triglyceride deposition and normal hepatic histology compared to C57BL/6 demonstrating that SR-BI plays an important role in the development of NAFLD. Glucose tolerance test (GTT) revealed that scarb1$^{-/-}$ mice responded much better to glucose administration showing that the lack of the SR-BI is not correlated with diabetes. Kinetic and gene expression analyses suggested reduced *de novo* fatty acid synthesis in scarb1$^{-/-}$ mice. Furthermore, adenosine monophosphate-activated protein kinase (AMPK)-stimulated hepatic FFA catabolism was reduced in these mice, leaving direct dietary lipid uptake from plasma as the major modulator of hepatic lipid content.
Conclusions: Our data suggest that scarb1$^{-/-}$ mice fed a western-type diet for 24 weeks accumulate CE- and ApoE-rich HDL of abnormal density and size. The elevated HDL-ApoE levels inhibit plasma LpL activity, blocking the clearance of triglyceride-rich lipoproteins and preventing the shuttling of dietary lipids to the liver.

PP6

**INVESTIGATING THE ROLE OF GSK3-BETA KINASE AS A MOLECULAR TARGET FOR MYOCARDIAL INFARCT SIZE REDUCTION BY USING NOVEL PHARMACOLOGICAL INHIBITORS.**

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Background: Mitochondrial Permeability Transition Pore (mPTP) is considered to be the end-point target of cardioprotection. Glycogen synthase kinase 3 beta (GSK3β) is implicated in multiple signal pathways involved in cardioprotection against isch/ rep and is proposed to be an upstream of mPTP. However, the results on the role of GSK3β are controversial and the number of GSK3β pharmacological inhibitors is limited. Previously, we have proven that the administration of indirubin-3 monooxime (BIO), belonging to a class of GSK3β inhibitors, reduces the infarct size in rabbits and increases the phosphorylation of GSK3β(S9).
Purpose: We sought to determine the role of GSK3β as a molecular target for infarct size limitation by administrating 4 novel indirubins, as GSK3β inhibitors with different affinity.
Methods: New Zealand white male rabbits were randomized into 5 groups and subjected to 30min myocardial ischemia and 3hours reperfusion (Control group) and the following additional interventions in 2776 Group, 2777 Group, 2778 Group, 2779 Group and BIO Group which were respectively treated with 0.0146 mmoles*Kg-1 of GSK3β inhibitors MLS2776, MLS2777, MLS2778, MLS2779 and BIO, given intravenously at the 20th min of isc. After the end of rep the % ratio infarct (I) to risk (R) ratio was estimated. Subsequently, additional animals were subjected to the same protocol up to the 10th min of rep where the hearts were excised and snap-frozen for GSK3β assessment. Additionally, mitochondria were isolated from the left ventricle of normal rabbits, in order to investigate a direct effect of the compounds on the mPTP through Calcium Retention Capacity (CRC) Assay.
Results: Infarct size was significantly reduced in all groups (%I/R: 9.1±3.1, 26.8±2.9, 10.2±1.9, 28.5±4.0, and 33.1±2.6, respectively, vs 49.5±3.9 Control, $p<0.05$). Groups 2776 and 2778 had significantly limited infractions compared with 2777, 2779 and BIO groups. Increased phosphorylation of GSK3β(S9) were obtained in groups 2776, 2777 and BIO in agreement with the in vitro results ($p<0.05$). Furthermore treatment of the compounds on isolated mitochondria showed no effect on CRC, indicating that the compounds do not exert their protective effects through direct inhibition of mPTP opening.
Conclusion: We conclude that phosphorylation of GSK3β at Ser9 does not result in infarct size limitation and GSK3β inhibition does not seem to be the end-effector of cardioprotection.

PP7

**ESCALATING LOW DOSES OF Δ⁹-THC DURING ADOLESCENCE AFFECT MOTOR ACTIVITY INDUCED BY KETAMINE OR D-AMPHETAMINE IN ADULT RATS**

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Background and objectives: *Cannabis* is one of the most widely used illicit recreational drugs especially among adolescents. Several studies have reported that Δ⁹-tetrahydrocannabinol (Δ⁹-THC), the main psychoactive component of the plant, may influence the sensitivity to other "club drugs" or psychostimulants. The purpose of the present study was to evaluate the motor effects of ketamine and d-amphetamine in adult rats that were exposed to low doses of Δ⁹-THC during adolescence.
Methods: Adolescent male and female Sprague-Dawley rats were pretreated with escalating low i.p. Δ⁹-THC doses twice daily from PND35 to PND45 (0.3mg/kg PND35–37; 1mg/kg PND38–41; 3mg/kg PND42–45) or vehicle. During adulthood (PND>75): A) One subset of rats was injected with an acute i.p. dose of d-amphetamine (1mg/kg), which acts as an indirect dopaminergic agonist, or saline and open-field motor activity in habituated rats was recorded for 60min and B) in a different subset of rats motor behavior was monitored in habituated rats after an acute i.p dose of ketamine (25mg/kg), which acts as a non-competitive NMDA receptor antagonist, or saline.
Results: A) Adolescent Δ⁹-THC pretreatment dampens the motor effects of d-amphetamine during adulthood in both male and female treated rats, as deduced by a reduced horizontal and vertical activity, in comparison with vehicle treated rats. B) Δ⁹-THC exposure during adolescence induces an abolishment in motor activity exhibited by ketamine only in adult male rats compared with vehicle-treated rats. On the contrary, Δ⁹-THC-treated female rats exhibited increased horizontal activity following ketamine administration during adulthood as compared with vehicle-treated rats.
Conclusions: The present findings show a strong impact of adolescent low-dose Δ⁹-THC pretreatment on adult motor activity induced by d-amphetamine and ketamine. These effects suggest that adolescent Δ⁹-THC exposure dampens the motoric responses to these psychostimulants later in adulthood, especially in males.

PP8

**OLEUROPEIN-INDUCED CYP-DEPENDENT DRUG METABOLISM**

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The main constituent of olive, oleuropein, is isolated from the olive leaves and olive oil. The activation of nuclear transcription factor PPARα has a beneficial effect on the cardiovascular function. Peroxisome proliferator-activated receptor alpha (PPARα) is expressed in the liver, kidneys, muscles and adipose tissue and its activation has essential roles in lipid homeostasis. The present study focused on the role of PPARα activation in xenobiotic metabolism and specifically, on the cytochrome-catalysed drug metabolism. For this purpose, SV129 wild type mice were treated with oleuropein for 6 weeks (3g food pellet /day + 0,3 g sucrose, 100mg/day). Similar treatment protocol was followed in P*parα*-null mice. The data of this study revealed that long term treatment with oleuropein significantly induced *Cyp1a1/2*, *Cyp1b1*, *Cyp3a14/25*, *Cyp2c29/44*, *Cyp2d22*, *Cyp2e1* hepatic expression and also the transcription factors CAR, PXR and RXR. These oleuropein-induced effects were mediated by PPARα activation, because no changes were observed in P*parα*-null mice following a long-term treatment with oleuropein. In conclusion, oral long-term oleuropein administration appears to activate PPARα, which in turn induces several major CYP isoforms critical for the metabolism of the vast majority of prescribed drugs, precarcinogens, toxic agents and carcinogens. The present findings indicate the determinant role that oleuropein profoundly has as a food supplement in pharmacotherapy, pharmacotoxicity and carcinogenesis.

PP9

**PYRAZOLOPYRIDINES AS NOVEL CYSTATHIONINE BETA SYNTHASE INHIBITORS**

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Cystathionine beta synthase (CBS) is a pyridoxal phosphate (PLP) dependent enzyme that contains also heme-b as a cofactor. CBS condenses serine and homocysteine to cystathionine and participates in the enzymatic production of gas Hydrogen Sulfide (H2S). Cystathionine ß-synthase (CBS) among with Cystathionine .-lyase (CSE) and 3-Mercaptopyruvate Sulfurtransferase (3-MST) are responsible for hydrogen sulfide biogenesis in mammalian tissues. Hydrogen sulfide is a signalling molecule that belongs to the gas transmitter family and is increasingly recognized as an important biological and pharmacological mediator. The physiological functions of H2S include among others vasorelaxation and promotion of angiogenesis. Deficiency in H2S production is involved in various pathological disorders including inflammation and development of colon cancer. Genetics defects in CBS appear to be the leading cause of hereditary homocystinuria. Therefore, CBS activity and endogenous H2S production stand as promising pharmacological targets.
The aim of this study was to identify compounds that selectively bind to CBS enzyme and modulate its activity. The in-house library of natural products and synthetic analogues of University of Athens comprising ~2K molecules of exquisite structural originality was evaluated. A tandem screening approach based on the combination of virtual and experimental methodologies was utilized. Virtual screening was based on two complementary methods, namely 3D-molecular similarity and docking-scoring
calculations by utilizing ROCS and GLIDE software, respectively. Then, a consensus scheme was used for combining results aiming at selecting the top-ranked molecules.
The most promising candidates were evaluated in vitro using recombinant CBS and a colorimetric assay measuring the production of Hydrogen Sulfide. Screening afforded a number of compounds with interesting modulating properties against CBS and the IC50 values of the most potent inhibitors were calculated by dose-response curves.
Interestingly, the strongest effect was demonstrated by derivatives of the pyrazolopyridine scaffold. Docking calculations were subsequently undertaken to gain insight to the interactions of these compounds with the CBS active site.

PP10

***CETP* TaqI B POLYMORPHISM AND CHRONIC HCV INFECTION AND TREATMENT: A PRELIMINARY ASSOCIATION STUDY**

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Introduction - Aim: Cholesteryl ester transfer protein (CETP) is well known for its role in reverse cholesterol transport through its active involvement in the exchange of cholesterol esters and triglycerides between high density lipoprotein (HDL) and very low and low density lipoproteins (VLDL; LDL). There is, however, evidence suggesting that CETP may also be involved in the control of intracellular lipid metabolism, as it was shown to affect the formation of lipid droplets in an adipocyte cell line. As, in hepatocytes (that also express CETP), lipid droplets are an essential component of Hepatitis C Viral (HCV) propagation, we chose to examine the effect of the most well known common CETP gene polymorphism (*CETP Taq*IB) on chronic HCV infection and treatment.
Methods: We have genotyped the *CETP Taq*IB polymorphism in the DNA of 162 previously characterized patients with chronic HCV infection and 142 controls, with an established PCR-RFLP method. The *CETP* genotype frequencies between infected patients and controls, as well as between patients who achieved sustained viral response (SVR) and those who didn't, were compared with the $\chi^2$ test of independence. The association between *CETP Taq*I and plasma lipid parameters of chronic HCV patients at baseline was examined with ANCOVA. The interaction of the *CETP Taq*I polymorphism with ABCB1 G2677T/A and C3435T, previously genotyped in the same groups, was also tested.
Results: A non-statistically significant increase of *CETP Taq*I B2B2 genotypes was observed among controls compared to HCV patients, and among patients who achieved SVR compared to those who didn't. HCV-infected patients with the same genotype displayed a strong tendency for higher total plasma cholesterol levels ($p = 0.056$) compared to the other genotypes. *CETP Taq*IB appeared to interact with ABCB1 G2677T/A, as a previously published effect of the latter on chronic HCV infection was observed only among *CETP Taq*I B1 carriers ($p = 0.005$).
Conclusion: CETP may affect susceptibility to HCV infection but additional data are needed in order to characterize its effect.

PP11

## CIRCULATING EPIGENETIC BIOMARKERS IN THE WNT CARCINOGENETIC PATHWAY: PROGNOSTIC VALUE IN ADJUVANT AND METASTATIC COLORECTAL CANCER.

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Introduction: Wnt signaling plays an essential role in tumor initiation and progression as it is now obvious that many steps in its pathway are altered genetically or/and epigenetically during the process of carcinogenesis. Particularly, in colorectal tumorigenesis enacts a critical early event which primarily results from inactivating mutations in its crucial component, *APC* gene. In this study, we use cell-free circulating DNA from patients with early and advanced gastrointestinal tumors to analyze systematically the CpG methylation patterns in the gene promoters of three Wnt signaling components, *APC*, *SOX17* and *WIF.* Following, we correlate these patterns with patient prognosis and survival.
Materials and methods: Using Real-time Methylation Specific PCR, we examined the methylation status of these three gene promoters in the serum cell free DNA (sfDNA) extracted from i) 88 operable colorectal cancer patients (CRC) ii) 67 metastatic (mCRC) patients, and iii) 70 healthy volunteers. Results were correlated to patient clinicopathological findings.
Results: Our results show that *SOX17, WIF1,* and *APC* promoter hypermethylation is a frequent epigenetic event in adjuvant and metastatic colorectal cancer patients. *APC* was correlated with poorer prognosis and survival in both operable and metastatic disease, whereas *SOX17* was correlated with shorter survival only in metastatic CRC.
Conclusion: Further studies in a larger cohort of patients are required to further explore the prognostic significance of these three potential non-invasive epigenetic biomarkers in colorectal cancer.

PP12

## PHARMACOEPIGENOMICS OF TYPE 2 DIABETES MELLITUS: NO CORRELATION OF KCNJ11 DNA METHYLATION WITH HYPOGLYCEMIA IN SULFONYLUREA-TREATED PATIENTS

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Introduction-Aim: Sulfonylureas (SUs), a mainstay of Type 2 diabetes mellitus (T2DM) pharmacotherapy for over 50 years, are insulin secretagogues which act by blocking the ATP-sensitive potassium ($K_{ATP)}$ channels in pancreatic β-cells and thereby inducing insulin secretion. Potassium inwardly rectifier 6.2 subunit (Kir6.2), encoded by *KCNJ11* gene, makes up, together with sulfonylurea 1 receptor, the $K_{ATP}$ channel which consists the therapeutic target of SUs. Mild hypoglycemia is a frequent adverse event affecting many patients treated with these oral hypoglycemic drugs, being a serious drawback in patient adherence to therapy and everyday clinical practice. The incidence of SUs-related hypoglycemic episodes is influenced by co-medication, co-morbidity, age, sex, liver and renal function, as well as by genetic background. However, in our previous study, we have not found an association of *KCNJ11* E23K polymorphism to SU-hypoglycemia (Ragia et al., 2012). In the present study, a pharmacoepigenetic approach was applied in T2DM patients for the first time, investigating the correlation of *KCNJ11* DNA methylation status on SU-induced mild hypoglycemic events, as well as other patient characteristics and their pharmacogenomic profile.
Methods: Sodium bisulfite-treated genomic DNAs of 171 SU-treated T2DM patients, including 88 that had experienced drug-associated hypoglycemia and 83 that had never experienced hypoglycemia, were analyzed for DNA methylation status of *KCNJ11* gene promoter via Real-Time Methylation Specific PCR. This patient group had also been previously genotyped for *KCNJ11* E23K polymorphism. The percentage of methylation in a sample was estimated using the formula *KCNJ11* methylation (%) $=1/1+2^{(-\Delta Ct)} \times 100$, where $\Delta Ct=Ct_u - C_{tm}$.
Results: Both groups (hypoglycemic and non-hypoglycemic T2DM patients) displayed similar DNA methylation status of *KCNJ11* gene promoter. Specifically, methylation was detected in 67/83 (80.1%) hypoglycemic and in 76/88 (86.4%) non-hypoglycemic T2DM patients ($p=0.319$). Methylation status was not associated to *KCNJ11* E23K genotypes, or any of the other patient characteristics including

age, gender and previous treatments. Additionally, the interaction of *KCNJ11* promoter methylation* E23K genotypes was not associated with sulfonylurea-induced hypoglycemia.
Discussion: The present data suggest that *KCNJ11* DNA methylation status does not influence the incidence of hypoglycemic events in SU-treated T2DM patients and is not related to their pharmacogenetic background.

PP13

**EFFECTS OF CHRONIC METHAMPHETAMINE TREATMENT ON BRAIN STRUCTURE AND FUNCTION IN THE RAT**

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Background and Objectives: Methamphetamine (MA) is a widely abused drug with devastating health effects. MA users consume MA frequently and for prolonged periods of time. We wished to study the effects of extended MA exposure on brain structure and function. To this end, we treated rats with MA for four months and we performed volumetric, in vivo and in vitro imaging, and behavioral measures.
Methods: Rats were treated daily with 4 mg/kg MA (low dose, LD MA) or 8 mg/kg MA (high dose, HD MA) or vehicle (saline) i.p. for four months. At the end of the treatment period, one group of rats was perfused with fixative and their heads were scanned at 21T MRI, for the study of MA effects on brain volume. Another group underwent *in vivo* [$^{18}$F]FDG micro Positron Emission Tomography (uPET), to determine regional brain glucose metabolism (rBGluM). A third group underwent *in vivo* [$^{11}$C]raclopride uPET imaging to determine dopamine D2 receptor availability and their fresh-frozen brains were used for in vitro measures of dopamine receptor and transporter levels. Motor activity in the open field was assessed on a weekly basis and novel object recognition was evaluated towards the end of treatment.
Results: MA treatment increased rBGluM in primary and higher order somatosensory areas and decreased rBGluM in rhinal cortices, hippocampus, and subiculum. In particular, HD MA decreased rBGluM in the tail of the striatum and produced a uniform decrease in the volume of the striatum. MA treatment decreased dopamine transporter and receptor levels and impaired novel object recognition performance and open field behavior.
Conclusions: This rat model of prolonged MA exposure reproduces structural and functional changes of the brain observed in addicts and helps further examine the mechanisms behind MA-induced neurotoxicity.

PP14

**CB2 RECEPTOR AGONISM DECREASES COCAINE-INDUCED CONDITIONED PLACE PREFERENCE AND MOTOR ACTIVITY**

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Background and Objectives: Studies have shown the involvement of cannabinoid (CB) receptors in the behavioral effects of psychostimulants. However, very few have investigated the respective role of CB2 receptors. This study was conducted to determine the role of CB2 receptors in cocaine's rewarding effects. To this end, we studied the effects of CB2 receptor agonist JWH-133 on acquisition and expression of cocaine-conditioned place preference (CPP) and on cocaine-stimulated motor activity. The effects of CB2 receptor antagonist AM630 pre-administration we also studied.
Methods: Cocaine CPP was performed with an unbiased design in a three-compartment apparatus, with 1 habituation day, 8 days of conditioning with alternating drug and vehicle injections, and 1 test day. Cocaine-stimulated motor activity was studied with the open field test.
Results: Neither JWH-133 nor AM630 induced CPP or CPA, in contrast to cocaine that induced robust CPP. JWH-133 decreased acquisition and expression of cocaine CPP and also decreased cocaine-stimulated motor activity. Pre-administration of AM630 completely blocked all the effects of JWH-133.
Conclusions: CB2 receptor agonism or antagonism has no rewarding or aversive properties *per se* in the CPP paradigm. The endocannabinoid system contributes, via CB2 receptors, to the primary rewarding properties of cocaine, to the expression of cocaine x environment associations, and to the stimulatory motor effects of cocaine.

PP15

**SCREENING FOR ANTIDEPRESSANT PROPERTIES WITH THE FORCED SWIM TEST: THE CASE OF FEMALES**

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Depression is twice more common in women than in men and sex differences have been reported in antidepressant treatment. However, most antidepressant tests, including the Forced swim test (FST) has been developed for male rats. Recent studies report serious discrepancies regarding sex differences in the FST and the duration of analyzed behaviors. In the present study, we compared the effects of sertraline, a selective serotonin reuptake inhibitor on male and female rats in relation to the phases of the estrous cycle.
FST in rats is performed in two consecutive days (15 and 5 min swim sessions separated by 24h) and females were used in all combinations of the phases of the estrous cycle. Male and female Wistar rats were subjected to the FST procedure and were treated with sertraline (10 mg/kg and 40 mg/kg, 3 i.p injections in 24 hours) or vehicle. Immediately after the second FST session, trunk blood was collected and gonadal hormones were assayed with RIA. Moreover, we performed a systematic review of the relevant literature and a frequency analysis on collected data from identified studies, reporting on male and female FST behavior.
Two-way ANOVA for immobility duration revealed that females exhibited higher levels of immobility than males and the sex difference was alleviated following antidepressant treatment (sex x treatment interaction $p<0.05$). The effect of the estrous cycle on the FST baseline performance of females is small, but the phase of the estrous cycle significantly affects the response to the antidepressant. Sertraline at both doses enhanced swimming in both sexes (treatment effect $p<0.05$), but the high sertraline dose enhanced climbing in females particularly in proestrous and diestrous phases of the cycle ($p<0.05$). Sertraline decreased testosterone levels only in males (sex x treatment interaction $p<0.05$), whereas high sertraline dose enhanced progesterone levels in proestrous and diestrous II females.

In conclusion, we propose that standard FST procedures and scoring methods should be used on both males and female animals, whereas the phase of the cycle should be taken under consideration. Furthermore, dose-dependent studies should be designed, when screening for new antidepressants in both sexes.

PP16
**SUSTAINED AROMATASE INHIBITION: BRAIN EFFECTS IN BOTH SEXES**

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Aromatase inhibitors, which are widely used in the clinic for the treatment of estrogen-dependent cancers, have been associated with psychiatric effects ranging from mania to depression. In the present study, we investigated the behavioral and neurochemical effects of aromatase inhibition on male and female rats.
Male and female adult Wistar rats were sham-operated or gonadectomized. Three weeks later, they received a chronic treatment of the aromatase inhibitor letrozole (1 mg/kg) or vehicle (i.p., once a day for one week) and they were subjected to the open field test (10 min) and to the standard forced swim test (15min FST pretest and 24 hours later, 5min FST test). Immediately after the second FST session, rats were killed and the hypothalamus, the hippocampus and the prefrontal cortex (PFC) were isolated. Aromatase activity in the hypothalamus was determined. The hippocampus and PFC were analyzed for monoamine and aminoacid analysis with high performance liquid chromatography (HPLC-ED).
Females were overall more active and explorative than males in the open field test (sex effect: $p<0.05$), whereas gonadectomy eliminated this sex difference. In the FST, females exhibited overall higher immobility levels than males. Interestingly, letrozole enhanced immobility in gonadectomized male and female rats, in comparison to sham-operated rats (interaction of surgery x treatment $p<0.05$); a finding indicative of enhanced "depressive-like" symptomatology in rats lacking both peripheral- and brain- derived estrogens. Letrozole inhibited aromatase in the brain, since aromatase activity in the hypothalamus was markedly decreased in letrozole- treated rats. Serum testosterone levels were markedly enhanced in female sham-operated rats treated with letrozole ($p<0.001$). Aromatase inhibition did not influence aminoacid's levels. Letrozole treatment affected noradrenaline levels, as well as dopamine and serotonin turnover ratios in the hippocampus and PFC. Sex differences, as well as gonadectomy and stress effects were also apparent.
Behavioral effects of aromatase inhibitors can be attributed to the inhibition of estrogen synthesis in the brain and could be further associated with serotonergic and dopaminergic changes in brain regions involved in depression. Importantly, the present data suggest a possible role of estrogen depletion in the development of affective disorders in post-menopausal women treated with aromatase inhibitors.

PP17
**GABAPENTIN AS AN ADDICTION TREATMENT: AN EVIDENCED- BASED STUDY**

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Backround/ Objective: Gabapentin is an anticonvasculant medication that effects neuronal transmission in a presynaptic level. It has been proven to increase GABAergic tone, thus affecting glutamergic transmission. Its role in the treatment of various substance addictions is being studied for several years. The objective of this study is to review the efficacy of gabapentin in treating alcohol, cocaine, opioids, nicotine, methamphetamine and cannabis dependence.
Methods: A customized literature search was performed dating from 2003 to 2015, using the keywords: gabapentin, addiction, withdrawal and treatment. It included clinical trials, controlled clinical trials, randomized controlled clinical trials, clinical studies, multicentered studies, observational studies, comparative studies, meta-analyses and reviews.
Results: From the data we gathered, it appears that gabapentin is associated with better treatment outcomes in alcohol and opioids addiction. In general, it alleviates withdrawal symptoms, improves sleep quality and reduces both the substance craving and the possibility of a relapse. It had dose-dependent effects on the alcohol addiction treatment and was mainly used in the treatment of opioid dependence during the withdrawal period due to its analgesic effects. On the contrary, gabapentin proved ineffective in the treatment of cocaine addiction. It did not have any effect on cocaine usage and substance craving. There were a few studies regarding nicotine and methamphetamine dependence with controversial results. In the case of cannabis we found one study, with positive results.
Conclusion: Gabapentin is a well-tolerated medication with few reported side-effects. It holds promise as an effective treatment for alcohol and opioids addiction as a combination therapy or monotherapy. Further research needs to be conducted, not only, in order to clearly establish the specific role of gabapentin in those addiction treatments and in clinical care, but also, so as to evaluate its efficacy in the treatment of cannabis, nicotine and methamphetamine dependence, where little evidence exists.

PP18
**THERMAL WATER AND COSMETIC**

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Introduction: According to the Greek mythology water is a primary vital element, which is related with vegetation, the fertility of the land, health and wellness. Many of the sources known to date were associated with deities and attributed medicinal properties. Hercules with the encouragement of the goddess Athena, used to bathe in the hot springs of Thermopylae and Edipsos, to be cured by the hardships and recover forces after each feat. The goddess Artemis was the protector of the thermal waters and she used to bathe in caves together with the nymphs that accompanied.
Purpose: During the recent years the thermal waters are increasingly used to replace all or part of the water in the recipes of cosmetics. The most important and most popular properties of the thermal water in the skin are presented.
Material and Methods: Relevant medical research was sought through the review of the literature. The sources were cross-checked for their validity and force.
Results: Salts, minerals and trace elements that are contained in thermal water offer unique properties to the skin. These properties find application in cosmetic and dermatological products, hydration, treatment of oily skin and acne, the treatment of atopic dermatitis in children and adults, eczema, psoriasis, burns, healing problems and sensitive skin. Water is the key ingredient of cosmetics and is contained at rates up to 100%.
Conclusions: Comparative study of various thermal waters and clinical research on the effect of water on the skin itself, prove their quality and the experience of centuries is confirmed: the thermal waters has strong perspective in the cosmetics field. That is in what we must invest in our country, with the tens of thermal baths, tracing traces of millennia of culture using means of modern science.

PP19
**MONOCLONAL ANTIBODIES AGAINST NERVE GROWTH FACTOR IN PAIN MANAGENT**

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Background/ Objective: Neuropathic pain and chronic pain constitute a serious problem to the daily medical practice and to the community, due to the fact that only palliative care is provided which slightly improves the patients' quality of life.Today, the role of monoclonal antibodies against the neural growth factor is being studied for pain management.Nerve growth factor (NGF) particiPatesin prenatal nerve growth, immune system function and mechanisms of pain. Several studies in pre- clinical and clinical level are trying to assess the efficacy of NGF inhibitors in pain management. The aim of this study is to present the NGFbiochemical pathwaysinvolving pain and the results of monoclonal antibodies targeting NGF on pain management.
Methods: A literature review of current studies was performed in medical databases, concerning the role of NGF in pain and NGF monoclonal antibodies in pain management.
Results:In a biochemical level NGF acts by binding to two receptors: the tropomyosin receptor kinase A (TrkA), and the p75 neurotrophin receptor (p75NTR). Monoclonal antibodies against NGF have been introduced in clinical trials regarding osteoarthritis, neuropathic and chronic low back pain. Tanezumab is the most well studied antibody in a pre- clinical and clinical level, compared to fulranumab andfasinumab. Although,a positive outcome in WOMAC index for arthritis and in pain management in general has been reported by the majority of the studies using tanezumab, a small impact on clinicalpractice and a moderate statistical significance was observed. Tanezumab has a dose-dependent effecton the clinical outcome. However, serious adverse effects (rapidly progressing osteoarthritis and osteonecrosis) appeared following the dosage increase, resulting in putting on hold all phase 3 clinical trialsin 2010. Trials are continued today, under certain safety criteria.
Conclusions: The data we gathered indicate that monoclonal antibodies against NGF inserts a promising target for novel treatment approaches. However, it appears that tanezumab is not well tolerated, especially in high doses.Further research needs to be conducted in order to overcome the current problems of the treatment and enhance its clinical efficacy.

PP20
**CHRONIC ADMINISTRATION OF THE SYNTHETIC CANNABINOID HU-210 INDUCES DOWNREGULATION OF THE CB1 CANNABINOID RECEPTOR IN RAT RETINA**
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Introduction: Endogenous and synthetic cannabinoids have been shown to protect the retina, in different models of neurotoxicity, via CB1 receptor (CB1R) activation. The use of cannabinoids as therapeutics in retinal disease is presently investigated at the preclinical level. Chronic exposure of the synthetic cannabinoid HU-210 has been shown to induce downregulaton of the CB1R in several brain regions. The aim of this study was to investigate whether subchronic or chronic administration of HU-210 leads to downregulation of the CB1R in rat retina.
Methods: Sprague-Dawley rats were treated with HU-210 (25, 50 μg/kg) by intraperitoneal injection (i.p), daily for 14 days (chronic administration) or for 4 days (subchronic administration). In order to study the acute effects of HU-210, rats were injected with vehicle for 13 days and a single dose of HU-210 (50μg/kg) on day 14. Immunohistochemical studies and western blot analysis were performed to examine the downregulation of the CB1 receptor in the different groups. A polyclonal antibody raised against the CB1 receptor was employed (1:300, Abcam).
Results: The CB1R immunoreactivity is localized mostly in the ganglion cell layer, and less in the inner plexiform, inner nuclear and outer plexiform layers. A decrease of CB1R immunoreactivity in the ganglion cell layer was observed after 4 or 14 days of HU-210 (50μg/kg) administration. Similar results were obtained with western blot analysis. The lower dose of HU-210, 25μg/kg, did not provide any statistically significant decrease in CB1R immunoreactivity, in any of the time points studied, nor did HU-210 (50μg/kg) administered acutely.
Conclusions: The results of the present study provide information regarding the distribution of the CB1R in rat retina. Furthermore, they show for the first time that HU-210, administered subchronically or chronically, downregulates the CB1R in rat retina in a dose dependent manner. Studies are in progress to examine this phenomenon in a disease model of retinopathy.

PP21
**LONG-TERM SIDE EFFECTS OF SSRIs AND SNRIs: MAPPING THE PHARMACOLOGICAL EVIDENCE**
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Introduction: Selective serotonin re-uptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) are widely used antidepressant agents. Although their acute adverse effects have been adequately studied, the side effects that probably arise after long-term use are less clearly described. Further, a controversial issue is the accurate definition of "long-term side effects" and the precise specification of a time-border between acute and chronic results. Aim of our study was to map the evidence in this field.
Methods: PubMed was searched using the terms 'SSRI', 'SNRI', or "antidepressant" and 'long-term side effects'. All studies presenting original data about the chronic use of SSRIs and SNRIs were included. Relevant references cited in these publications were also evaluated.
Results: The correlation of these drugs with weigh changes has been adequately documented; during the acute phase of the treatment a weigh decrease has been often observed, which is followed by weight gain. Described are also diabetes mellitus and metabolic syndrome, as well as the risk of decrease in bone density that results in increased prevalence of falls and fractures especially among elderly patients with osteoporosis. Other adverse events are pulmonary hypertension, cardiovascular disorders, hypertension or hypotension, fatigue, cognitive impairment and alopecia. Acute but insisting side effects that tend to be considered as chronic are sexual dysfunction, insomnia, hypersomnia and other sleep disorders. Highly alarming is the potential connection of serotonergic antidepressants with breast and ovarian cancer, although there is still controversy over that issue. Antidepressants have been also accounted for incidents of suicidal behaviour, with the risk of suicidality (ideation, events, attempts or preparation of suicide) supposed to be higher among children and adolescents, but stable among adults. Some side effects can be considered among long-term due to their tendency to worsen during extended use. These include hyperidrosia, night sweats, nightmares, tremor, aggressiveness and a synergic action of SSRIs or SNRIs with antiplatelet drugs (mostly aspirin and clopidogrel) that possibly increases bleeding risk.
Conclusion: Long term side effects contribute to increased morbidity, mortality and a loss of therapeutic gains. Given the necessity of chronic treatment of SSRIs and SNRIs further long-term clinical trials are needed focusing on the safety issues of these drugs.

PP22
**THE MECHANISTIC SIGNATURE OF ATYPICAL ANTIPSYCHOTICS: THE PHARMACODYNAMIC PROFILE**
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Introduction: Atypical antipsychotics have unique binding profiles by targeting neurotransmitter receptors. The individual pharmacodynamic profile elucidates mechanistic properties of functions and adverse effects.
Methods: Data concerning the binding profiles of antipsychotic agents have been searched in PubMed using the terms "binding profile OR pharmacodynamic", "antipsychotics", "lurasidone", "brexpiprazole". In addition Ki values have been searched in the Psychoactive Drug Screening Program Ki Database.
Results: Risperidone and ziprasidone are strong D2/3 antagonists, whereas aripiprazole and brexpiprazole act as partial agonists. Antipsychotic shave lower affinities for other dopamine receptors, but risperidone, clozapine and brexpiprazole are strong D4 antagonists. Clozapine, olanzapine and risperidone are strong H1 antagonists, whereas other histamine receptors are not targeted. Brexpiprazole, lurasidone and aripiprazole are strong 5-HT1A partial agonists, whereas other antipsychotics have weaker effects. 5-HT2A/2B and 5-HT2C blockade are common targets of atypical antipsychotics. Lurasidone and brexpiprazole are the most potent 5-HT2A and the least potent5-HT2c antagonists. Risperidone is also a strong 5-HT2A/2B/2Cantagonist. Lurasidone is the strongest 5-HT7 antagonist. Clozapine and olanzapine are unique by being partial agonists on muscarinic receptors. Atypical antipsychotics are also antagonists on alpha1/2 adrenergic receptors and they could act directly or indirectly on other receptors, such as nicotinic and norepinephrine/serotonin reuptake (Table 1).
Conclusions: The central dopaminergic system is essential target of antipsychotic agents. Atypical antipsychotics aim selectively the mesolimbic pathway without disrupting the mesocortical and nigrostriatal pathways, in order to maximize their efficacy and safety. 5-HT2A/2B antagonism and D2/D3 partial agonism are mediators of "atypicality", the lack of extrapyramidal symptoms. In addition 5-HT1A agonism and antagonism of 5-HT7, adrenergic and muscarinic receptors could have anxiolytic, antidepressive, antimanic and cognitive effects. However, complex pharmacodynamic profiles, such as muscarinic, adrenergic, H1 and 5-HT2c antagonism may cause cardiometabolic and autonomic side effects.

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| | D1 | D2 | D3 | D4 | H1 | 5-HT1A | 5-HT2A | 5-HT2B | 5-HT2C | 5-HT7 | M1 | M3 | a1 | a2A | a2B | a2C | Other |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Clozapine | + | + | + | ++ | +++ | + | ++ | +++ | ++ | ++ | +++ | ++ | +++ | ++ | ++ | ++ | NMDA, GABA |
| Olanzapine | ++ | ++ | ++ | ++ | +++ | | +++ | ++ | ++ | ++ | ++ | ++ | ++ | + | ++ | ++ | |
| (Nor)quetiapine | + | + | + | | +++ | ++ | ++ | | + | ++ | + | + | ++ | + | + | ++ | NET |
| Risperidone | + | +++ | +++ | +++ | +++ | + | ++++ | ++ | ++ | +++ | | | +++ | ++ | ++ | +++ | |
| Aripiprazole | | +++ | +++ | + | ++ | +++ | ++ | ++++ | ++ | ++ | | | ++ | ++ | ++ | ++ | SERT |
| Brexpiprazole | + | ++++ | +++ | +++ | ++ | ++++ | ++++ | +++ | + | +++ | | | +++ | ++ | ++ | ++++ | SERT, NET |
| Ziprasidone | + | +++ | +++ | ++ | ++ | ++ | ++++ | ++ | ++++ | +++ | | | ++ | + | ++ | ++ | SERT, NET |
| Lurasidone | + | +++ | | | | +++ | +++ | | + | ++++ | | | ++ | ++ | | +++ | nicotinic (?) |

**Table 1: Pharmacodynamic profile of common atypical antipsychotic drugs Quetiapine is** demonstrated along with norquetiapine, a metabolite of the drug with distinct binding profile. Antagonism and inverse agonism are indicated by red color whereas partial agonism by green. The number of crosses and the color intensity are correlated to the association constant.
100 < Ki < 1000: + weak association
10< Ki < 100: ++ moderate association
1< Ki < 10: +++ strong association
1 > Ki: ++++ very strong association

PP23
**ILLICIT DRUG AND ALCOHOL USE AMONG UNDERGRADUATE AND POSTGRADUATE MEDICAL STUDENTS IN NORTHERN GREECE**

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Introduction: Medical students may be at a higher risk for substance use and this can lead to unfavorable patterns and affect their professional life in the future. The aim of this study was to investigate the prevalence of illicit drug use and the correlations with demographic factors and alcohol use among medical students in Northern Greece.
Methods: 657 undergraduate and postgraduate medical students from the Faculty of Medicine of the Aristotle University of Thessaloniki participated in the study completing a self-administered web-based survey. The survey included questions regarding: i) prevalence of illicit drug use (e.g marijuana, heroin, cocaine, ecstasy, amphetamines, LSD, mephedrone) ii) the CAGE questionnaire, a widely used screening test for potential alcohol problems iii) a question investigating binge drinking behavior. All statistical analyses were performed using SPSS v. 22.0.
Results: Overall, 657 students completed the survey. The mean age of the participants was 22.2±2.3 years and 90% were undergraduate students. Lifetime use of illicit drugs was 23,9% (157/657). The mostly used substance was marijuana (21%) followed by cocaine (2.3%), ecstasy (2.3%), LSD (2%), ketamine (2%), and amphetamines (1.8%). 6.2% of the participants scored positive in the CAGE scale and 22.2% in the binge drinking scale. In the multivariate model illicit drug use was significantly correlated with number of cigarettes ($p<0.001$), binge drinking ($p<0.001$) and also the study level (undergraduate/postgraduate, $p=0.016$). There was no significant correlation with the other factors (gender, study year, CAGE).
Conclusions: Approximately one quarter of the participants reported use of illicit drugs and several factors are independently associated. Further research is needed to investigate the prevalence, the motivation and also the impact of this risky behavior among medical student population.

PP24
**THE USE OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS) AMONG THE MEDICAL STUDENTS OF THE ARISTOTLE UNIVERSITY OF THESSALONIKI**

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Introduction: Nonsteroidal anti-inflammatory drugs (NSAIDs) are a class of drugs that are widely used in the general population to alleviate symptoms such as headache and fever.
Aim: Through this research we attempted to collect data on the use of NSAIDs by the Medical School students of the Aristotle University of Thessaloniki. We studied the individual character of the use, the most common causes, the occurrence of side effects and the possible addiction.
Methods: The survey was conducted through questionnaires distributed and completed anonymously and voluntarily from a sample of 200 medical students studying in various years. The sample consist-

ed of 108 women and 92 men, aged 18-37 years. After the collection of the questionnaires, statistical processing and data analysis of the sample were conducted.
Results: The vast majority of the students have consumed NSAIDS at least once during their studies. First in consumption is paracetamol (77.5%), followed by mefenamic acid (30%). The principal causes of using are headache (58.5%) and fever (40%). In terms of side effects, the most common are gastrointestinal disorders (19.5%) while 78% have not mention any side effect. 63.5% resort to NSAIDs only when symptoms become intolerable, while 1.5% are dependent on them.
Conclusion: The rate of use of NSAIDs in medical students is quite high and there are addictive behaviors even in this young and healthy population. It is noteworthy that many wish to use an alternative form of treatment of the symptoms, such as acupuncture and homeopathy.

PP25
**THE USE OF CANNABIS AND CANNABINOIDS FOR MEDICAL PURPOSES**
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Introduction: The use of cannabis for its medicinal properties has been presented for centuries and has been incre
easing in recent years. Cannabinoids are the primary active componentsof cannabis and they can be divided into 'natural' cannabinoids, such as Δ9-tetrahydrocannabinole (THC) and cannabidiol and 'synthetic' cannabinoids, such as dronabinol, nabilone and nabiximols.
Aim: The purpose of this review is to summarize the literature regarding the medical uses of cannabis and canabinoids.
Methods: Review of the literature concerning evidence from animal experiments and clinical trials involving humans about the medical use of cannabis and cannabinoids.
Results: Cannabinoids are more effective for the relief of nausea and vomiting than other medications like phenothiazines and antihistaminic drugs, as well as for certain types of chronic pain and weight loss in patients with acquired immune deficiency syndrome (AIDS). Cannabinoids have also been studied for their anticancer effects, some of which are inhibition of tumor angiogenesis, decrease of cancer cell migration and temporarily relief of symptoms. Nevertheless, no firm conclusions are yet possible, due to the small number of clinical trials in humans. Currently is being researched in clinical trials, the use of nabiximols for the treatment of pain and spasticity in multiple sclerosis.
Conclusion: Over the last years there has been an important advance in the knowledge of the endocananabinoid system and it is believed that although the potential risks from the use of cannabis, cannabinoid pharmaceuticals combined with other drugs, may lead to better methods of clinical uses.

PP26
**SEARCHING THROUGH NATURE'S ARSENAL FOR POTENTIAL**
**BETA AMYLOID PEPTIDE INHIBITORS**
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Neurodegenerative disorders such as Alzheimer's Disease (AD) and Parkinson's Disease are the most prevalent neurodegenerative diseases and have a major health and medical care cost impact. Their main characteristic is severe neuronal loss, and they are associated with abnormal accumulation and aggregation of disease-specific peptides and inclusion bodies in selected brain regions. In case of AD, beta amyloid peptide (Aβ)-containing plaques and intraneuronal neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein are the two main neuropathological lesions. Therefore, understanding the aggregation mechanism and how to inhibit aggregate formation is crucial and will have a major impact on health. In view of the suggested link between oxidative stress and neurodegeneration, endogenous antioxidants or dietary derived compounds may be prime candidates for anti-aggregation compounds preventing aggregation of Aβ.
In this study, we demonstrate an integrated approach towards the evaluation of the antiamyloidogenic activity of isolated components from endemic plants of Greece as putative aggregation inhibitors for the prevention of AD. Several components from *Crocus sativus* L., have been fully characterized and screened for forming noncovalent complexes with the Aβ peptide. These *Crocus sativus* L.-derived bioactive constituents are trans- and cis-crocin-4 (TC-4, CC-4), crocin-3 (TC-3, CC-3), crocin-2 (TC-2, CC-2), and other crocetin mono- and bis-ester glycoside compounds. The screening of these compounds for binding with Aβ was carried out by nano-electrospray ionization Mass Spectrometry (MS), where the formation of 1:1 noncovalent complexes of Aβ with TC-2, CC-2, TC-3, CC-3, TC-4 and CC-4 was observed. In addition, *in vitro* screening was supplemented with cell viability assays using differentiated neuronal SH-SY5Y cells. At the cellular level, trans-crocetin and TC-4 did not appear to have any toxic effects at concentrations up to 10μM at 24 and 72 h. Moreover, trans-crocetin and TC-4 at concentrations 0.1-10 μM appear to modestly enhance cell proliferation at 24 h. The real-time monitoring of these interactions facilitates the design and development of novel anti-aggregation compounds. It will also provide the basis for *in vivo* studies employing the *double* $APP_{swe}/PSEN_{1dE9}$ *Tg* mouse model, which have increased Aβ production in AD-relevant brain areas.

PP27
**EFFECTS OF THE INDUCIBLE NITRIC OXIDE SYNTHASE INHIBITOR AMINOGUANIDINE IN DIFFERENT RAT MODELS OF SCHIZOPHRENIA**
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Rational: Schizophrenia is a severe chronic mental health disease. Current medications have demonstrated some utility in treating the positive but not the negative symptoms or cognitive dysfunction of schizophrenia. Nitric oxide (NO) is considered as an intra-cellular messenger and its implication in schizophrenia is documented. Specifically, overproduction of NO is linked to this pathology. Thus, reduced NO activity might be beneficial in schizophrenia.
Aims: The present study has investigated the ability of the inducible NO synthase inhibitor (iNOS) aminoguanidine (AG) to counteract schizophrenia-like behavioural deficits produced by the N-methyl-D-aspartate (NMDA) receptor antagonist ketamine and the mixed dopamine (DA) $D_1/D_2$ receptor agonist apomorphine in rats.
Methods: AG's ability to reverse ketamine and apomorphine-induced memory deficits was assessed using the novel object recognition task (NORT). The ability of AG to counteract ketamine-induced social withdrawal was evaluated using the social interaction test.
Results: AG (25 and 50 mg/kg) counteracted ketamine (3 mg/kg) and apomorphine (1 mg/kg)-induced performance deficits in the NORT. Conversely, AG (50-100 mg/kg) failed to attenuate the ketamine (8 mg/kg) induced social withdrawal in the social interaction test.
Conclusions: Our findings illustrate that AG reversed recognition memory deficits induced by NMDA receptor blockade or DA receptor agonism in rats. On the other hand, AG did not counteract ketamine-induced social isolation.

PP28

**ESCALATING LOW-DOSE Δ⁹-THC TREATMENT DURING ADOLESCENCE IMPAIRS PSYCHOMOTOR FUNCTIONS AND INDUCES REGION-SPECIFIC NEUROCHEMICAL ALTERATIONS IN A SEX-DEPENDENT MANNER**

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Introduction: Experimental studies have shown that the main psychoactive component of cannabis, delta-9-tetrahydrocannabinol (Δ⁹-THC), during adolescence can trigger long-term behavioral and neurochemical alterations in adulthood.

Aim: Evaluation of the behavioral effects of adolescent escalating low-dose Δ⁹-THC administration in adult male-female rats and region-dependent neurochemical indices.

Methods: Between PND35 and PND45 adolescent rats received escalating low-dose Δ⁹-THC i.p. twice daily (0.3mg/kg PND35–37; 1mg/kg PND38–41; 3mg/kg PND42–45) or vehicle. On PND75: a*)* open-field motor activity and behavioral motor habituation were recorded b) neurochemical variables were assessed in the prefrontal cortex (PFC), hippocampus (HIPP), nucleus accumbens (NAC) and dorsal striatum (DS).

Results: a) Δ⁹-THC-treated male rats showed increased reactivity to novelty versus vehicle, while female treated rats exhibited opposite effects. Δ⁹-THC exposure had an impact on male rats concerning the behavioral motor habituation pattern.

b) In the PFC, DA turnover ratio was decreased in male Δ⁹-THC-treated rats, versus vehicle. In contrast, DA turnover ratio was higher in HIPP, while a similar trend observed in NAC, as deduced by higher DOPAC tissue levels. In the DS, decreased DA tissue levels were observed in Δ⁹-THC-treated male rats compared to vehicle.

Δ⁹-THC-treated females did not show any dopaminergic alteration in PFC and HIPP as compared to vehicle. In the Nac, decreased DOPAC tissue levels were observed, while striatal DA tissue levels were increased.

Concerning serotonergic activity, an increased 5-HT turnover ratio was observed in PFC of male Δ⁹-THC-treated rats, while an opposite effect was found in HIPP. In the Nac, an increased 5-HT turnover ratio was assessed, while an increase in striatal 5-HT tissue levels was observed in Δ⁹-THC-treated males versus vehicle.

In Δ⁹-THC-treated females, a reduced 5-HT turnover ratio was assessed in the PFC and NAC, while hippocampal 5-HIAA tissue levels were increased as compared to vehicle. It appears that the dopaminergic and serotonergic status was different between males and females, following adolescent Δ⁹-THC exposure.

Conclusions: Present findings show a sex-dependent effect of adolescent low-dose Δ⁹-THC treatment on adult motor stimulation and habituation. A region-specific and sex-dependent effect on monoaminergic activity was observed in prefrontal and subcortical brain regions, following Δ⁹-THC exposure.

PP29

**ANTI-ATHEROSCLEROTIC AND CARDIOPROTECTIVE PROPERTIES OF CROCUS SATIVUS L. AQUEOUS EXTRACT IN APOE$^{-/-}$ DEFICIENT MICE**

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Purpose: To assess the effects of saffron aqueous extract (SFE), on the development and stability of atherosclerotic lesions in diabetic diet apolipoprotein-E deficient (Apo-E$^{-/-}$) mice and its cardioprotective properties under ischaemia(isc)/reperfusion (rep) injury.

Methods: 1) 40 male Apo-E$^{-/-}$ mice were fed high fat diabetogenic diet for 12 weeks and randomized to receive either SFE 30 mg/kg (group SF1,n=10), 60mg/kg (SF2,n=10), 90 mg/kg (SF3,n=10) or water for injection (WFI) (control group, COG, n=10) for 4 weeks, followed by glucose tolerance test and calculation of the respective area under the concentration vs time curve (AUC). After euthanasia, lipid and glucose profiles in blood samples were assayed, while in aortic tissue specimen the mean plaque area (MPA), the relative plaque content of elastin and collagen, and the thickness of fibrous cap (FC) were measured. 2)male Apo-E$^{-/-}$ mice following normal diet were subjected to 30 min isc followed by 3 hrs rep, randomized to receive: a) WFI (COG,n=6):, b) orally SFE (SFE,n=6,60 mg/kg, 4-weeks). Ischemic area and area at risk were measured. Ischemic myocardial tissue was also collected from Apo-E$^{-/-}$ mice, subjected to the same interventions up to 10th min of reperfusion (n=6) for the expression of p-eNOS, eNOS, p-Akt, Akt, p-p42/p44, p42/p44, iNOS, IL-6 and MnSOD.

Results: MPA was smaller in SF groups than COG. Notably, SFE treatment seemed to promote plaque stability. Atherosclerotic lesions in SF groups showed increased collagen ($p<0.001$) and elastin ($p<0.001$) content, thicker FC ($p=0.023$) and reduced number of Internal Elastic Lamina ruptures per mm of arterial girth ($p<0.001$) compared to COG. All SFE doses considerably ameliorated glucose levels and AUC. SFE reduced infarct size (16.8±1.1 vs 45.3±2.0,$p<0.0001$) compared to COG. eNOS, Akt and p-44/p-42 phosphorylation, upregulation of MnSOD and reduced IL-6 and iNOS expression was observed in SF groups.

Conclusions: SFE exerted anti-diabetic, anti-atherosclerotic effects and promoted plaque stability in Apo-E$^{-/-}$ mice in a dose-dependent manner. SFE protected against isc-rep injury through activation of RISK pathway, upregulation of MnSOD and dowregulation of IL-6 and iNOS. Thus, it can be suggested that SFE may be a possible application as natural medicine or food supplement with prospective anti-atheromatic and cardioprotective effects.

PP30

**THE ROLE OF ERDJ5 FACTOR IN PRION DISEASE PATHOGENESIS**

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Introduction: The involvement of the family of protein disulfide isomerases (PDIs) in neurodegenerative diseases has been thoroughly studied (2,3) in relation to protein misfolding and aggregation, which have been found to induce massive endoplasmic reticulum (ER) stress (1). ERdj5 is a member of the PDI family that is required for ER export of terminally misfolded proteins in the Endoplasmic-Reticulum- Associated protein Degradation (ERAD) by reducing the disulphide bonds (4,5).

Materials and Methods: An experimental animal model has been used

to study the involvement of the ERdj5 factor in the pathogenesis of prion diseases,. In particular, 18 female mice, strain 129SV, were separated in two groups (6). The first group comprised of 10 mice, in which the ERdj5 was silenced; these were homozygotes for the silenced gene (knock out, KO, ERdj5$^{0/0}$). The second served as a control group and was comprised of 8 wild type (wt) animals. The animals were challenged intraperitoneally with scrapie infectious material, derived from the RML model of scrapie disease in mice (7). To monitor post-inoculation disease progress, the animals were placed under close surveillance and under went further clinical examination, which included body weight measurement and estimation of their mobility and perception.At the endpoint of the disease, mice were sacrificed and tissues were collected for further investigation.
Results: At the beginning of the clinical phase, knock-out animals had developed significant early symptoms in comparison to the control group. The disease in KO animals appeared to be quite aggressive: animals lost nearly 50% of their original weight, were fully attenuated with an intense appearance of involuntary horror. The wild-type animals showed the first visible disease symptoms much later and, they generally exhibited similar but less severe symptoms.
Discussion: In conclusion, it can be safely stated that the disease developed later in wild type animals, with a significantly shorter clinical phase and less severe symptomatology. Our data demonstrates that presence of ERdj5 implies a potential protective role due to its participation in proper protein folding.

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PP31

**VACCINATION: A POSSIBLE TREATMENT AGAINST ADDICTION?**

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Introduction: Substance abuse and addiction are serious social problems of the last century. Today, various pharmacological and non-pharmacological treatments are available for addiction therapy. Recently, an immunization treatment, based on vaccination against addiction has been introduced to the research field. The objective of this study is to review the efficacy of this treatment on cocaine, methamphetamine, opioids and nicotine addiction.
Methods: A literature search of current studies was performed in medical databases, concerning the use of vaccination to treat substance use disorders.
Results: Vaccination treatment of addiction is based on the principle of reducing or eliminating the amount of the substance passing the blood/ brain barrier, thus, reducing its positive reinforcement, facilitating abstinence and preventing relapse. To date, vaccines have been created against nicotine, morphine/heroin, cocaine, and methamphetamine, with research primarily focused on cocaine and methamphetamine vaccines. Most of the studies still remain in a preclinical level and present controversial results. Although anti- nicotine and anti- cocaine vaccinations are currently being evaluated in clinical trials, there are still doubts about the level of their efficacy but optimism is prevailing. Vaccination against opioids and methamphetamine still remains in an early developmental stage, which indicates promising results.
Conclusion: Vaccines are generally well tolerated in the clinical trials that are being conducted, with mild side effects being reported in the case of cocaine. It appears from the preliminary data of the existing studies that vaccination to treat drug use disorders is a promising solution. However, there are still many difficulties to overcome and further research needs to be conducted in order to enhance the efficacy and to establish the boundaries of the treatment.

PP32

**CYTOTOXICITY OF RE/ENROFLOXACIN COMPLEX IN TWO DIFFERENT CANCER CELL LINES**

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Quinolones, synthetic antibacterial drugs for the treatment of diverse infections, inhibit the activity of the bacterial DNA-topoisomerase II, essential for the replication and transcription of DNA. Due to homology with eukaryotic DNA-topoisomerase II, quinolone derivatives, including metal complexes, have been studied for possible anticancer activity. In a previous work, we developed quinolone derivatives with tricarbonyl Rhenium(I) with increased binding capacity with DNA. Among these derivatives, [Re(CO)$_3$(enrofloxacin)(imidazole)] (***Re-erx***) exhibited increased inhibitory activity against the eukaryotic topoisomerase II, as compared with the enrofloxacin (***erx***) alone, a 2nd generation fluoroquinolone. We also developed the radiotracer complex [$^{99m}$Tc(CO)$_3$(erx)(im)](***$^{99m}$Tc-erx***) by substitution of Re with the gamma emitting radionuclide Technetium-99m ($^{99m}$Tc), in order to investigate its uptake in K-562 human erythroleukemia cells. Incubation of the tracer ***$^{99m}$Tc-erx*** with K-562 cells over 20 hrs led to a time-dependent uptake of the complex. Subsequent study of its subcellular distribution showed uptake of ***$^{99m}$Tc-erx*** in the mitochondrial fraction, indicating mitochondria, and maybe mitochondrial DNA, as possible target of ***$^{99m}$Tc-erx.*** In the present work, we studied the possible cytotoxicity of ***$^{99m}$Tc-erx*** in two different cancer cell lines. K-562 cells and the U87-MG glioblastoma cells were incubated with different concentrations of ***Re-erx*** και ***erx*** and assessed for cell growth and viability after 48 hrs. In both cell cultures, increased cytotoxicity was observed, after incubation with ***Re-erx,*** as compared with ***erx*** alone. More specifically, in K-562 the $IC_{50}$ for ***erx*** was ~30 X $10^{-6}$ M and for ***Re-erx*** was a little lower, ~17 X $10^{-6}$ M. However, in case of ***Re-erx*** in K-562 cells, cell death was up to 76%. In U87-MG cells, the $IC_{50}$ for ***erx*** was ~90 X $10^{-6}$ M and was much lower for ***Re-erx (***~17 X $10^{-6}$ M), but without difference in the observed cell death for the used concentrations. In conclusion, the ***Re-erx*** quinolone derivative exhibits increased cytotoxic effect in the two used cancer cell lines compared to the quinolone (***erx)*** alone, and could be considered as an attractive lead compound for the development of new antineoplastic agents.

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PP33

**EFFECT OF ZOLEDRONIC ACID ON BUCCAL MUCOSA**

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Introduction: Zoledronic acid (ZA), a nitrogen-containing bisphosphonate has a cytotoxic effect on oral epithelial cells. The aim of our study was to investigate the effects of ZA on buccal mucosal cells and on VEGF expression.
Methods: Twenty female Wistar rats were divided into two subgroups of 10 animals, the Experimental Group and the Control Group. ZA was administrated per os to animals at a dose of 0.05mg/kg b.w. /week for 13 weeks. After euthanasia, the mandible of the animals was removed and the tissues were processed for electron microscopy and immunohistochemical examination of VEGF.
Results: ZA caused a decrease of the epithelial cell layers in 20% of the mandibular tissues and 40% of them appeared pyknotic nuclei, indicating the mechanism of apoptosis. The basal lamina was ruptured and had decreased number of hemidesmosomes in 70% of them and in 60% the dermis had an extensive edema. VEGF expression was found weakly (+) positive in 40% of the sections.
Discussion: ZA has a potent apoptotic effect on buccal epithelial cells and in the structure of dermis. The increased expression of VEGF in the experimental group indicates the remodelling of the mucosal architecture.

PP34

**THE EVALUATION OF A PATIENT'S QUALITY OF LIFE AFTER A MAJOR THORACIC SURGERY OPERATION AND ITS CORRELATION WITH THE TYPE OF POST-OPERATIVE ANALGESIA**

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Introduction: Major thoracic surgery procedures constitute a standard method of diagnosis and/or therapy against lung, mediastinal cancer and other non malignant manifestations of the respiratory system. These are, a) Open Thoracotomies and, b) VATS (Video Assisted Thoracic Surgery) procedures. Such patients' recovery and rehabilitation depend directly from the applied postoperative analgesia, with purpose to determine an optimum and long term quality of life.
Background: Our "database" consists of approximately 100 patients who underwent major thoracic surgery procedure in a 6 month period of time (between Aug.2015 and Jan.2016) at the "Thoracic Surgery Dpt." of "Theageneio" Cancer Hospital, Thessaloniki.
Methods: The two principal classification groups include the abovementioned operative methods, while each group presents three subgroups, depending on the type of postoperative analgesia: 1) Thoracic epidural analgesia (level $T_3$-$T_8$) through intravenous injection of opioids (Morphine), 2) Thoracic epidural analgesia (level $T_3$-$T_8$) through combined intravenous injection of opioids (Morphine) and local intramuscular injection of Ropivacaine and, 3) Combined intravenous injection of Tramadol and Non Steroidal anti inflammatory drugs (NSAID's). The "Quality Of Life" (QoL) Questionnaires used for the determination of the QoL Index are, i) "EORTC QLQ-C30", ii) "SF-36", iii) "EQ-5D" and iv) the "VAS – Visual Analog Scale" for pain.
Results: Each classified patient answers the Questionnaires in specific time intervals such as, immediately postoperatively, the day of discharge, after 1 month, and 4 months period. The differentiation in their answers is the key point to extract important information about their postoperative health evolution. A detailed questionnaire evaluation follows, both individually and in groups, according to the subgroup of each patient's pain treatment, a combined study which is applied in this form for the first time.
Conclusions: The measure of a "QoL" index is widely taken into account as one of the most accurate indicators of a patient's health evolution. Our points of reference are the patients who undergo a major thoracic surgery procedure and their postoperative period. The results supply us with significant information, regarding eventual complications and discomforts, mainly postoperative pain, kinetic or respiratory discomfort, e.t.c., while they indicate us towards a thorough realization of each patient's "follow up" individually.

PP35

**THE ROLE OF ATP-SENSITIVE POTASSIUM CHANNELS IN ANGIOGENESIS**

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Introduction-Aim: ATP-sensitive potassium channels ($K_{ATP}$) are known to be activated by endothelial cell growth modulators such as C-type Natriuretic Peptide (CNP) and Hydrogen Sulfide ($H_2S$). However, their exact role in angiogenesis is far from clear. We therefore decided to test a) whether direct $K_{ATP}$ activation can elicit angiogenesis, b) whether CNP is capable of inducing angiogenesis, and c) whether Vascular Endothelial Growth Factor (VEGF) angiogenic effects depend on $K_{ATP}$ activation.
Methods: We tested our hypotheses in Chick chorioallantoic membranes (CAM) *in vivo* and in Human Umbilical Vein Endothelial cells (HUVECs) and bEnd.3 mouse endothelial cells *in vitro*.
Results: In CAM, direct activation of $K_{ATP}$ by Nicorandil and SG-209 or indirect activation by CNP resulted in increased angiogenesis, similar in magnitude to that evoked by VEGF. These CAM responses were abolished by the $K_{ATP}$ inhibitors Glibenclamide and 5-Hydroxydecanoate (5-HD). In bEnd.3 cells *in vitro*, Nicorandil, SG-209, VEGF and CNP all increased cell proliferation and migration. VEGF and CNP also induced capillary-like network formation in Matrigel by HUVECs. Glibenclamide or 5-HD effectively abrogated all these endothelial responses *in vitro*. bEnd3 and HUVEC cell migration and tubular network formation in Matrigel were largely reduced by siRNA-mediated knockdown of the inwardly rectifying potassium channel ($K_{ir}$) 6.1 subunit.
Conclusion: From these data we conclude that $K_{ATP}$ activation, dependent on $K_{ir}$ 6.1 expression, may underpin endothelial angiogenic responses to a number of disparate endothelial stimuli, such as $H_2S$, VEGF and CNP. It follows that impairment of these beneficial endothelial effects of $K_{ATP}$ by sulfonylureas (well-known blockers of $K_{ATP}$ channels) may underlie the clinical observation that diabetic patients treated with these drugs show a worse clinical outcome after a cardiac ischemic event and exhibit loss of ischemic postconditioning. $K_{ATP}$ channels may therefore play a more important role than previously thought in angiogenesis and endothelial cytoprotection *in vivo*.

PP36

**STRUCTURAL DETERMINANTS OF ACTIVATION OF THE CORTICOTROPIN RELEASING FACTOR RECEPTOR**

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Corticotropin releasing factor (CRF) is a 41-amino acid peptide that is present in many regions within the central nervous system, where it functions as a neurotransmitter/neuromodulator. The CRF plays a key role in anxiety and depression, mostly through its interaction with the type 1 CRF receptor ($CRF_1R$). So far many non-peptide $CRF_1R$-selective antagonists displayed antidepressant and anxiolytic properties in preclinical studies, without, however, any of them being available for clinical use. A major obstacle to the synthesis of novel non-peptide $CRF_1R$ drugs is the lack of information about the molecular mechanisms underlying $CRF_1R$ activation and its antagonism by non-peptide ligands, despite the structural information for this receptor obtained in a recent crystallization study. $CRF_1R$ is a plasma membrane protein, consisting of seven transmembrane domains (TMs) that link their extracellular with their intracellular regions. The binding of CRF to the extracellular regions of $CRF_1R$ triggers activation-associated conformational changes of receptor that are transmitted through the TMs to its intracellular regions and are responsible for the production of a biological effect. Non-peptide antagonists interact with the TMs of $CRF_1R$ and allosterically decrease CRF binding and receptor activation. In the present study we started elucidating the molecular mechanisms of $CRF_1R$ activation, and its allosteric antagonism by non-peptide ligands by determining the interactions between TM residues of $CRF_1R$, their rearrangements during receptor activation, and the role of non-peptide ligands in these interactions. These studies will advance the receptor-based design of novel $CRF_1R$-selective non-peptide drugs, with potential anxiolytic and antidepressant properties.

PP37

**ELUCIDATION OF THE ROLE OF THE SECOND EXTRACELLULAR LOOP OF $CRF_1$ RECEPTOR IN SIGNALING**

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The type 1 receptor ($CRF_1R$) for the corticotropin releasing factor (CRF) plays an important role in many physiological and pathophysiological processes, by activating different signaling pathways. The $CRF_1R$ is a plasma membrane receptor, which couples to G-proteins and consists of seven membrane-spanning segments connected by three intracellular and three extracellular loops. Previously we have shown that the CRF and the CRF related peptide, sauvagine, interact with the second extracellular loop of $CRF_1R$. In the present study we found that Ala mutation of Trp259 and Phe260, which are located in the second extracellular loop of $CRF_1R$, largely decreased sauvagine but not CRF ability to stimulate cAMP accumulation. In marked contrast these mutations significantly reduced the binding affinities of both peptides. These results suggest that among the interactions of Trp259 and Phe260 of $CRF_1R$ with CRF and sauvagine, only those between sauvagine and Trp259 /Phe260 are important for $CRF_1R$-mediated stimulation of cAMP accumulation. These results led us to hypothesize that Trp259 and Phe260 act as molecular switches to regulate distinct signaling pathways after their interaction with different ligands. Experiments under way will confirm this hypothesis by determining the signaling pathways that are stimulated by the interaction of different CRF-peptides with Trp259 and Phe260 of $CRF_1R$. These studies are of high significance because they will advance the rational receptor-based design of novel signaling selective $CRF_1R$-ligands. These molecules could be used to uncover the molecular mechanisms underlying the role of $CRF_1R$ in many physiological and pathophysiological processes.

PP38

**PHARMACOLOGICAL PROPERTIES OF NOVEL GNRH ANALOGUES**

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The gonadotropin releasing hormone (GnRH) is a hypothalamic 10 amino acid peptide, which interacts with the GnRH receptor (GnRH-R) and stimulates the secretion of gonadotropins from pituitary gland. Gonadotropins play an important role in the function of the reproductive system. In addition to its important role in the function of the reproductive system, the GnRH modulates the proliferation of several tumor cells that express the GnRH-R. Continuous administration of GnRH to prostate cancer cells expressing the GnRH-R resulted in reduction of their proliferation. This study aims to design, synthesize and pharmacologically evaluate some new GnRH analogues. Some of the new GnRH analogues displayed significant binding affinity and reduced the proliferation of GnRH-R-expressing cells. These molecules will form the basis for the development of novel more effective, stable and long-acting GnRH analogues, with improved binding affinity and efficacy.

PP39

**DEVELOPMENT OF NOVEL ANALOGUES OF NEUROTENSIN**

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Neurotensin (NT) is a 13 aminoacid peptide, (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu), which displays a wide spectrum of biological actions in the central nervous system (CNS) as well as the periphery andplays a significant role in many physiological and pathophysiological conditions. Most of theactions of NT are mediated through its interaction withthe type 1 NT receptor (NTS1 receptor). The development of small NT analogues, proteolytically stable, with improved pharmacokinetic profiles would enrich the pharmaceutical armoire against several diseases, such as those of the CNS. To develop such molecules we started designing and synthesizing NT analogues based on the structure of the C-terminal hexapeptide fragment of NT [NT(8-13)]. This fragment is an obvious lead compound for development because it contains the necessary structural requirements to bind to NTS1 receptor and

elicit biological effects. Given that theNT(8-13) is rapidly degraded by peptidases the linear and cyclic NTS analogues synthesized in this studycontain modifications in the structure of NT(8-13)that improve metabolic stability. To pharmacologically evaluate the novel NT analogues we performed homologous competition radioligand binding studies using $^{3}$H-Neurotensin and membrane preparations of the HT-29 cell line which endogenously express the NTS1 receptor. This study revealed that among 15 NT analogues, the analogues, NT5, NT6 and NT8 bound to NTS1 receptor with relatively high affinity. These new analogues will put the basis for the development ofa new generation of NT molecules with improved pharmacological properties.

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