

POSTER ABSTRACTS

CARDIOVASCULAR PHARMACOLOGY

P01-01

SYMPATHETIC POTENTIATING ACTION OF 5-HT IN DIABETIC RATS TREATED WITH FLUOXETINE

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Diabetes Mellitus is a common disease in Western countries, usually associated with a higher incidence of depression. It is well-known that serotonin deficiency is the underlying cause of depression and, also, it has been suggested a possible role for 5-hydroxytryptamine (5-HT) in the pathophysiology of diabetic complications. Our previous results have suggested that 5-HT exerts a presynaptic inhibitory action on electrically induced pressor responses in normoglycaemic and diabetic rats. However, in normoglycaemic rats treated with fluoxetine, 5-HT also exerts a potentiating effect. The aim of this work was to study the 5-HT types/subtypes receptor involved in the serotonergic effect on the pressor responses obtained by electrical stimulation (monophasic pulses, the duration and intensity of 1 ms supramaximal increase in the frequencies 0.1, 0.5, 1.0 and 5.0 Hz) of sympathetic outflow from the spinal cord in diabetic pithed rats treated with fluoxetine (10 mg/kg, dissolved in drinking water over 14 days). Diabetes was induced by a single s.c. alloxan injection. Intravenous infusions of 5-HT (5–80 µg/kg per min) reduced the pressor effects obtained by electrical stimulation in diabetic pithed rats treated with fluoxetine; inhibition reproduced by 5-carboxamidotryptamine, 5-CT (5 µg/kg per min), a selective 5-HT₁ agonist. Whereas, the 5-HT₂ receptor agonist, alpha-methyl-5-HT (5 µg/kg pre min), enhanced the pressor responses, abolished by ritanserin (1 mg/kg), and R96544 (1 mg/kg), selective 5-HT₂ and 5-HT_{2A} receptor antagonists, respectively. These results suggest that, in diabetic rats treated with fluoxetine, 5-HT induces potentiating actions of the pressor responses obtained by electrical stimulation of sympathetic outflow, via 5-HT_{2A} receptor activation.

P02-01

VISFATIN IMPAIRS ENDOTHELIUM-DEPENDENT RELAXATION IN MICROVESSELS: ROLE OF NAMPT AND NAD

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Introduction: Visfatin, also known as extracellular pre-B-cell colony-enhancing factor (PBEF) and nicotinamide phosphoribosyltransferase/visfatin (Nampt), is an adipocytokine enhanced in type 2 diabetes mellitus and obesity, and positively associated with vascular damage. Here, we investigated whether visfatin can directly induce endothelial dysfunction in isolated mesenteric microvessels from both male Sprague-Dawley rats and omentum samples from patients undergoing abdominal surgery.

Results: Pre-incubation of rat microvessels with visfatin (50 and 100 ng/ml) did not modify noradrenaline contractions (1 pM–30 µM), as determined using a microvessel myograph. However, visfatin (10–100 ng/ml) concentration-dependently impaired the relaxations to acetylcholine (ACh; 100 pM–3 µM), without interfering with the endothelium-independent relaxations to sodium nitroprusside (1 nM–3 µM). In cultured human umbilical vein endothelial cells, visfatin (10–100 ng/ml) concentration-dependently stimulated NADPH oxidase activity, as

determined by lucigenin-derived chemiluminescence. Indeed, the relaxation to ACh impaired by visfatin (50 ng/ml) was restored by the NADPH inhibitor apocynin (10 µM). Additionally, the Nampt inhibitor APO866 prevented the NADPH oxidase activation and the impaired relaxations by visfatin. Accordingly, the product of Nampt activity nicotinamide mononucleotide (100 nM–1 mM) stimulated endothelial NADPH oxidase activity and concentration-dependently impaired ACh-induced relaxations. In human mesenteric microvessels pre-contracted with 35 mM KCl, endothelium-dependent relaxations to bradykinin (1 nM–3 µM) were also impaired by visfatin and restored upon co-incubation with APO866.

Conclusions: Visfatin directly induces endothelial dysfunction through a mechanism involving NADPH oxidase stimulation and relying on Nampt enzymatic activity.

P03-01

PROTOCOL FOR MEASURING ELECTRON MICROSCOPY IMAGES: PLASMATIC MEMBRANE SARCOPLASMIC RETICULUM

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Introduction: Due to the growing use of electron microscopy for scientific studies, it is necessary to create methodologies for measuring the images obtained, is our purpose to measure the plasmatic membrane (PM) sarcoplasmic reticulum (SR) apposition.

Material and Methods: We processed human and mice mesenteric arteries for obtaining images with an electronic transmission microscope (Jeol., JEM-1010, Japan). 60 k zoomed serial images from each cell were taken, mounted and measured with Inkscape freeware. We measured the cell PM perimeter and the apposing SR at 50 nm maximum separation. This allows us to calculate the apposition percentage and check the SR refilling. Which Fameli et al. said to be a minimum of 15% of PM.

Results: This method allowed us by the moment to measure five young (8–9 weeks old) mice cells (three from each) with percentages of 11.67, 16.38, 19.39, 25.22, 23.64, and three adults (75–75 weeks) mice cells (three from each) with percentages of 22.15, 14.67, 22.69.

Conclusions: This protocol allows us to do precise measurements of the PM-SR apposition and check that the majority of mice would be able to refill the SR to produce arterial contraction.

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P04-01

ROLE OF CARDIOVASCULAR DISEASE AND DRUG THERAPY ON HUMAN ENDOTHELIAL PROGENITOR CELL NUMBERS: IN VITRO EFFECT OF TGF-β1 PATHWAY BLOCKERS

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Introduction: Atherosclerosis has been linked to endothelial progenitor cell (EPC) depletion and anti-atherosclerotic drugs such as statins have

been shown to modulate this fact. In addition, some anti-inflammatory cytokines such as transforming growth factor beta (TGF β 1) may exert a protective effect on this cell type.

Material and Methods: EPC number *in vivo* was assessed by flow cytometry. EPCs were cultured from peripheral blood mononuclear cells from healthy donors by using the early-outgrowth protocol. Apoptosis was assessed by DNA fragmentation ELISA. Protein expression *in vitro* was determined by Western blot.

Results: We found a lower number of CD34+/CD144+ EPCs in CABG patients compared to valvular patients, despite a higher rate of cardiovascular drugs consumption. Moreover, numbers of CD34+/CD144+ EPCs remained unchanged in CABG patients who were treated with statins and angiotensin converting enzyme inhibitors (ACEIs). However, plasma from CABG patients decreased apoptosis in cultured early-outgrowth EPCs (EOCs) from healthy donors (day 7). This effect was abrogated by the transforming growth factor-beta 1 (TGF- β 1) pathway inhibitors SIS3 (Smad3 blocker) and SB-431542 (ALK-4 blocker). Interestingly, prior to apoptosis protection (day 4), plasma from CABG increased CD34 and CD144 expression in EOC cultures from healthy donors.

Conclusion: Enhancing the TGF- β 1 pathways Smad3 and ALK-4 is a potential Therapeutical strategy for future pharmacological interventions in order to promote EPC survival in cardiovascular disease.

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P05-01

SECRETOME ANALYSIS OF HUMAN ATHEROSCLEROTIC ARTERIAL TISSUE REVEALS POTENTIAL MARKERS OF PATHOGENESIS

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Background: The early detection and knowledge of the underlying mechanisms in cardiovascular diseases are essential. Secretome studies shows a much narrower protein concentrations dynamic range than plasma, as an *in vivo* situation. This study was aimed to discover proteins with key roles in atherosclerosis development, analyzing human arterial tissue (HAT) secretome and quantitative comparison of healthy and atherosclerotic tissue.

Methods: Secretome collection from tissue in culture was optimized and minimization of plasma proteins was checked by 2-DE. HAT secretomes were analyzed by LC-MS/MS in an Orbitrap. Three biological replicates of human atherosclerotic coronary arteries (AC), preatherosclerotic coronaries (PC) and mammary (M) secretomes were analyzed. Label-free MS/MS-based validation and quantification was performed by Scaffold and Sieve programs. The identified proteins were analyzed with Ingenuity Pathway Analysis (IPA) system.

Results: Sixty-four proteins were identified in the three replicates of at least one of the M, PC, AC groups, of which 14 secreted proteins have not been reported in plasma. By IPA, 15 molecules have been related to cardiovascular system development and function and the top network associated was cellular movement, cell death, cellular growth and proliferation. Quantitative comparison resulted in four proteins significantly released in higher amounts by mammary tissue: gelsolin, vinculin, lamin A/C and phosphoglucosyltransferase 5.

Conclusion: We highlight the relevance of tissue secretome as reflected by the identification of proteins secreted by HAT which were not reported in plasma. Proteins involved in inflammation, cell to cell adhesion and arterial aging processes had been identified as significantly regulated.

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P06-01

RETINOID X RECEPTOR (RXR) AGONISTS INHIBIT VASCULAR INFLAMMATION THROUGH PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-GAMMA (PPAR γ) UPREGULATION

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Background: The migration of leukocytes into inflamed tissues involves a cascade of molecular events regulated by cell adhesion molecules (CAMs) and chemokines. Retinoid X receptor (RXR), a member of the nuclear receptor superfamily, forms heterodimers with several nuclear receptors including PPAR γ and mediates many biological effects.

Objective: To characterize the functional role of RXR agonism in vascular inflammation.

Methods: Human umbilical arterial endothelial cells (HUAECs) were used for these studies. HUAECs were stimulated with TNF α (10 ng/ml) and some of them were incubated with different RXR agonists such as bexarotene and 9 cis Retinoic Acid (9 cis-RA) 20 h prior to TNF α stimulation, MCP-1 and GRO- α levels were determined by ELISA. In some experiments, cells were transfected with a RXR α specific siRNA to knockdown RXR α expression.

Results: In human endothelial cells, the RXR agonists, bexarotene and 9 cis-RA, inhibited TNF α -induced MCP-1 and GRO- α release in a concentration dependent manner. However, in cells transfected with RXR α siRNA, the inhibitory effect was abrogated. Furthermore, TNF α -induced RXR α and PPAR γ downregulation was attenuated by RXR agonists. Immunoprecipitation analysis revealed that RXR α effects were mediated by increased RXR α -PPAR γ interactions.

Conclusions: Taken together, our results suggest that the up-regulation of nuclear hormone receptors RXR α and PPAR γ seems to be involved in the protective anti-inflammatory effects exerted by RXR agonists.

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P07-01

EFFECT OF AGE AND PIOGLITAZONE ON HUMAN VASCULAR SMOOTH MUSCLE CELL PROLIFERATION

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Introduction: Although a growing body of evidence highlights the role of aging in vascular physiology and pharmacology, description of an age-dependent proliferative effect on human vascular smooth muscle cells is still lacking.

Material and Methods: Vascular smooth muscle cells were obtained from inferior mesenteric arteries, from patients who underwent abdominal surgery for colon cancer, appendicitis, inflammatory bowel disease or obesity. Proliferation was assessed by a BrdU incorporation kit. Apoptosis was measured by DNA fragmentation ELISA. Some experimental groups were coincubated with pioglitazone 100 mcM.

Results: Human vascular smooth muscle cells from aged patients showed a lower proliferative rate than the ones that were obtained from younger subjects, from 100 \pm 6.038% proliferative rate in younger than 20 years old to 62.49 \pm 8.675% in older than 75 years old (P < 0.01). However, pioglitazone did not exert a significant extent in this parameter. When apoptosis was assessed, no significant changes were found among different age groups.

Conclusion: Age is an important factor to modulate human vascular smooth muscle cell proliferation, rather than apoptosis. The effect of age is stronger than the one exerted by the antidiabetic drug pioglitazone. This finding may be important to design future age-tailored antiproliferative treatments for proliferative vascular diseases.

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P08-01

EFFECT ANTI-CD44 AND ANTI-CD62L ON HUMAN ENDOTHELIAL PROGENITOR CELLS: IMPLICATIONS FOR AN ALTERED VASCULAR FUNCTION IN CHRONIC MYELOPROLIFERATIVE NEO

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Introduction: Myeloproliferative neoplasms are chronic neoplastic disorders where an increased cardiovascular mortality takes place. Several studies link endothelial progenitor cell (EPC) dysfunction with vascular dysfunction and thrombosis. On the other hand, the cell adhesion molecules CD44 and CD62L have been shown to be upregulated in patients with myeloproliferative neoplasms.

Material and Methods: EPCs were cultured from peripheral blood mononuclear cells from healthy donors by using the early-outgrowth protocol and cultured on fibronectin-coated 6-well plates. Medium (MV-II microvascular) was changed at day 4 and number of colony forming units (CFU) was assessed at day 7. For the last 72 h, some cells were coincubated with anti-CD44 or anti-CD62L at 10 mcg/ml each one. Proliferative rate was assessed by a BrdU proliferation kit.

Results: Antibodies dramatically increased the number of CFU per plate from 3.5 (control) to 755 (anti-CD44) and 23.6 (anti-CD62L). However, they did not change the proliferative rate at a significant extent.

Conclusion: Although CD44 and CD62L have been implicated in hematopoietic stem cell survival, their inhibition increases CFUs in EPC cultures. Thus, these two molecules may mediate the EPC dysfunction that has been described in chronic myeloproliferative neoplasms and subsequent cardiovascular disease.

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P09-01

EFAVIRENZ INDUCES LEUKOCYTE RECRUITMENT *IN VIVO* THROUGH MAC-1/ICAM-1 INTERACTION

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Introduction: Highly active antiretroviral therapy (HAART) has been linked to the development of cardiovascular diseases. Efavirenz (EFV) is one of the most widely used antiretroviral agents, and thus cannot be ruled out as a causal agent of these side effects. We have demonstrated that EFV promotes leukocyte accumulation *in vitro* through Mac-1/ICAM-1 interaction. The present study was designed to confirm our data *in vivo*.

Methods: Leukocyte rolling, adhesion and emigration in mesenteric venules of anaesthetized rats were monitored using intravital microscopy. These parameters were determined 4 h after intraperitoneal administration of clinically relevant doses of EFV (15 µM) or methanol (vehicle). Adhesion molecules involved in EFV-induced responses were determined by intravenous pre-treatment of animals with antibodies directed

against rat Mac-1 (CD11b/CD18) or its endothelial ligand ICAM-1 (CD54). A one-way ANOVA + Newman-keuls analysis was performed, and statistical significance was **P < 0.01 (vs. vehicle), n=4 animals.

Results: Administration of EFV promoted a significant increase in leukocyte rolling flux (62.8 ± 4.2 vs. 29.8 ± 4.4**), adhesion (8.8 ± 0.8 vs. 2.0 ± 0.4**) and emigration (7.0 ± 1.1 vs. 0.6 ± 0.2**) with respect to that observed in vehicle-treated animals. Blocking antibodies against both subunits of Mac-1 (CD11b and CD18) or ICAM-1 (CD54) prevented the leukocyte recruitment induced by this drug.

Conclusion: Acute exposure to EFV induces leukocyte-endothelial cell interactions in rat mesenteric microvenules at clinically relevant doses *in vivo*. This process is mediated by the β2 integrin Mac-1, which interacts with its endothelial ligand ICAM-1. These results suggest that EFV is involved in the genesis of the cardiovascular diseases observed in HAART-treated patients.

P10-01

EPICATECHIN RESTORE ENDOTHELIAL FUNCTION IN DOCA-SALT HYPERTENSION: ROLE OF ENDOTHELIN-1, NADPH OXIDASE AND NRF2 PATHWAYS

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Introduction: Flavanols-rich diets have been reported to exert beneficial effects in preventing cardiovascular diseases, such as hypertension. Our present study was designed to examine whether chronic intake of epicatechin, the main dietary flavanol, prevents the DOCA-salt-induced hypertension and endothelial dysfunction.

Material and Methods: Rats were randomly divided into five groups: control, (-)-epicatechin (EPI10, 10 mg/kg), DOCA-salt, DOCA-salt-EPI2 (2 mg/kg) and DOCA-salt-EPI10 (10 mg/kg). Rats were daily administered by gavage for 5 weeks.

Results: The high dose of epicatechin prevented both the increase in systolic blood pressure and proteinuria induced by DOCA-salt. Plasma endothelin-1 and malondialdehyde levels and urinary isoprostaglandin F2α excretion, were found to be increased in animals of DOCA group. Epicatechin 10 mg/kg treatment reduced these parameters in DOCA-salt rats, having no effects on control rats. Aortic superoxide levels was enhanced in DOCA-salt group and abolished by both doses of epicatechin. However, only epicatechin 10 mg/kg reduced the raise in aortic nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase activity and aortic p47phox and p22phox gene overexpression found in DOCA-salt animals. Epicatechin increased the transcription of nuclear factor-E2-related factor-2 (Nrf2) and Nrf2 target genes in aortas from control and DOCA-salt rats. Epicatechin also improved the blunted endothelium-dependent relaxation to acetylcholine in phenylephrine precontracted aortic rings and increased the phosphorylation of both Akt and eNOS.

Conclusion: All these results suggest that a chronic treatment with epicatechin prevents hypertension and vascular dysfunction. Epicatechin prevent vascular oxidative stress by reducing ET-1 release, inhibiting NADPH oxidase activity and increasing Nrf2-driven antioxidant defences.

P11-01

IMPLICATION OF EXTRACELLULAR SIGNAL-REGULATED KINASE IN THE ACTIVATION OF HEAT SHOCK PROTEIN 27 OBSERVED AFTER NALOXONE-INDUCED MORPHINE WITHDRAWAL IN THE RAT HEART

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Introduction: Different drugs of abuse, such as morphine and/or its withdrawal, induce severe cellular stress situations that can cause a sudden change in the cellular environment, to which the cell is not prepared

to respond and there is an urgent need of cellular safeguards in the form of heat shock proteins (Hsps). In this study, we have investigated Hsp27 expression and its phosphorylation at Ser82 during morphine dependence and withdrawal and have evaluated the interaction between Hsp27 and ERK in the left ventricle.

Material and Methods: Dependence on morphine was induced by a 7-days sc implantation of morphine pellets. Morphine withdrawal was precipitated on day 8 by injection of naloxone (2 mg/kg, s.c.). In addition, rats were injected with SL-327, an inhibitor of ERK phosphorylation (100 mg/kg i.p.) or vehicle 1 h before naloxone. Rats were killed at different time points and Hsp27 expression and phosphorylation was determined by western-blot.

Results: Naloxone-precipitated morphine withdrawal increased the expression and phosphorylation of Hsp27 at Ser82 30, 60, 90 or 120 min after the injection of the opioid antagonist. However, there were not changes in Hsp27 phosphorylation in the morphine dependent group injected with saline. Moreover, pretreatment with SL-327, decreased the activation (phosphorylation) of Hsp27, suggesting that ERK triggers Hsp27 phosphorylation.

Conclusions: The present findings demonstrated that morphine withdrawal is capable of inducing the activation of Hsp27 in the heart and suggest that phosphorylation is closely linked and also dependent on the ERK pathway.

P12-01

TREATMENT WITH A NOVEL RICE BRAN ENZYMATIC EXTRACT IMPROVES CARDIOMETABOLIC RISK FACTORS IN OBESE ZUCKER RATS

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Introduction: Rice Bran Enzymatic Extract (RBEE) used in this study has several advantages such as its water-solubility and its enrichment in : mono- and polyunsaturated fatty acids, γ -oryzanol, L-arginine, tocopherols and tocotrienols. Our aim was to determine the effects of RBEE in the prevention of metabolic syndrome in Obese Zucker Rats (OZR).

Material and Methods: OZR were fed 1% and 5% RBEE supplemented-diet (OZ1 and OZ5, respectively), and their lean littermates (LZR) were fed at the same way (LZ1 and LZ5); simultaneously, OZR and LZR groups, fed standard diet, were used as control. Body weight, food and water intake, and systolic blood pressure (SBP) were weekly evaluated. After treatment, biochemical assays of serum glucose, triglycerides, total cholesterol, non-esterified fatty acids (NEFA) and nitrates were determined.

Results: RBEE treatment induced a significant SBP lowering in OZ1 and OZ5 (135.73 \pm 2.82 mmHg in OZ1, $P < 0.001$ and 142.39 \pm 1.39 mmHg in OZ5, $P < 0.05$) vs. OZR (153.13 \pm 3.21 mmHg). Five percent RBEE induced a substantial decrease in serum glucose levels of treated OZR (9.54 \pm 0.55 mM in OZ5 vs. 12.85 \pm 0.84 mM in OZR, $P < 0.05$), total cholesterol (5.00 \pm 0.26 mM in OZ5 vs. 6.33 \pm 0.33 mM in OZR, $P < 0.05$) and nitrates (28.78 \pm 2.97 μ M in OZ5 vs. 44.32 \pm 4.97 μ M in OZR, $P < 0.01$) whereas 1% RBEE was unable to alter the biochemical profile in these groups. NEFA values were modify by 5% RBEE (1.22 \pm 0.08 mM in OZ5 vs. 0.89 \pm 0.06 mM in OZR, $P < 0.05$).

Conclusion: These findings show RBEE as a supplement with nutraceutical properties, which may be useful in the prevention of complications associated to obesity.

P13-01

β -ADRENOCEPTOR COUPLING WITH CYCLIC NUCLEOTIDES FORMATION IN RAT AORTA AND SMALL MESENTERIC ARTERIES

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Introduction: The β_1 -, β_2 -, and β_3 -adrenoceptors (ARs) subtypes participate in regulating cardiovascular function and produce a vasorelax-

ation in blood vessels. The intracellular signaling pathways for β -ARs-mediated responses involve not only cAMP/PKA, but also endothelial nitric oxide (NO) release and the subsequent activation of cGMP/protein kinase G (PKG). The aim was to determine the β -AR subtype implicated in cAMP and cGMP formation in rat aorta and small mesenteric arteries (SMA).

Material and Methods: Cyclic nucleotides levels were determined employing different selective agonists and antagonists (1 μ M) on isoprenaline-induced stimulation.

Results: In rat aorta, isoprenaline (non-selective β -agonist) increased cAMP (313.66 \pm 30.47%, $n = 23$) and cGMP (256.43 \pm 27.83%, $n = 13$) levels (vs. basal). Propranolol inhibited these increases, CGP20712 (β_1 -selective) inhibited cAMP, whereas ICI1855 (β_2 -selective) and SR59230A (β_3 -selective) inhibited cGMP isoprenaline-induced stimulation. Dobutamine (β_1 -selective agonist) increased cAMP, whereas salbutamol (β_2 -selective) and CL316243 (β_3 -selective) augmented cGMP formation. In SMA, isoprenaline increased cAMP (303.66 \pm 32.31%, $n = 8$) to a greater extent than cGMP (174.63 \pm 19.00%, $n = 8$) levels (vs. basal). Propranolol, CGP20712 and SR59230A inhibited cAMP, while SR59230A inhibited cGMP accumulations; dobutamine increased cAMP and CL316243 augmented both nucleotides.

Conclusion: In the aorta, β_1 stimulation was coupled with an increment of cAMP, and the β_2/β_3 subtypes were involved in cGMP formation. In SMA, β_1 and β_3 -ARs were implicated in cAMP formation and β_3 -AR also provoked an increment in cGMP levels.

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P14-01

DIFFERENT CARDIOVASCULAR PROTECTIVE EFFECTS OF QUERCETIN ADMINISTERED BY ORAL OR INTRAPERITONEAL VIA IN SPONTANEOUSLY HYPERTENSIVE RATS

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Introduction: It has been showed that a quercetin-supplemented diet did not delay the onset or lessens the severity of cardiovascular complications that develop in spontaneous hypertensive rats (SHR) in contrast when quercetin was delivered via oral gavage. Therefore, we tested whether the administration procedure of this flavonoid affect its metabolite profile and antihypertensive activity.

Material and Methods: Forty SHR were randomly assigned to four experimental groups: (i) control group that received vehicle solution (1 ml of 1% methylcellulose) by gastric probe (ii) quercetin group that received a dose of 10 mg/kg by gastric probe (iii) quercetin group that received a dose of 10 mg/kg divided in two doses (5 + 5) by gastric probe and (iv) quercetin group that received a dose of 10 mg/kg by i.p. injection. Rats were treated daily for 5 weeks.

Results and Conclusion: The efficacy of single dose and two daily doses, in a long-term oral treatment was equally efficient, both restoring the impaired aortic endothelium-dependent vasodilatation and reducing blood pressure, heart rate and heart and kidney hypertrophy. These effects seem to be related to attenuation of vascular O_2^- production mediated by NADPH oxidase inhibition. However, the chronic administration by intraperitoneal via reduced in lesser extent than oral administration the systolic blood pressure but in this case that was not accompanied with an endothelial protection and antioxidant effects. The higher plasma methylated metabolites in oral administration could be the responsible in the beneficial effects of quercetin administrated orally.

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P15-01

ANTIHYPERTENSIVE EFFECTS OF THE PPAR- β AGONIST GW0742 IN DOCA-SALT HYPERTENSIVE RATS

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Introduction: The selective PPAR- β agonist GW0742 reduced blood pressure, improved endothelial dysfunction and reduced vascular proinflammatory and proatherogenic status in spontaneously hypertensive (SHR) by interference to angiotensin II signaling pathway. The chronic effects of GW0742 in deoxycorticosterone acetate (DOCA)-salt-induced hypertensive rats were analysed.

Material and Methods: Rats were divided into four groups: control, control-treated (GW0742, 5 mg/kg/day), DOCA-control, and DOCA-treated. Rats were daily administered by gavage for 5 weeks.

Results: Rats receiving DOCA-salt showed a progressive increase in systolic blood pressure (SBP) as compared to animals of the control group. The left ventricular weight index and the kidney weight index, endothelin-1 (ET-1) and malondialdehyde (MDA) plasma levels and 24 h urinary iso-PGF2 α excretion were significantly higher in DOCA-salt rats than in sham control rats. Endothelium-dependent relaxation to acetylcholine was impaired in aorta from DOCA group, and NADPH oxidase activity, and its subunits p47phox and NOX-4 gene expression were also increased. GW0742 induced a significant reduction (-13%) in SBP in DOCA rats but not in control rats. No significant changes were observed in renal and cardiac hypertrophy, systemic oxidative stress and endothelial dysfunction. Interestingly, GW0742 induced an endothelium- and PPAR β -independent vasodilatory effect in small mesenteric arteries, being without effects in endothelial dysfunction induced by ET-1.

Conclusion: GW0742 prevents the rise of blood pressure, without affecting the cardiac hypertrophy and functional vascular changes in DOCA rats. These results confirm the selectivity of PPAR β in vascular alterations mediated by angiotensin II, being without effect in that mediated by ET-1.

P16-01

MECHANISMS UNDERLYING THE IMPROVEMENT OF ENDOTHELIUM DYSFUNCTION INDUCED BY CHRONIC PPAR β ACTIVATION IN DIABETIC RATS

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Introduction: Endothelial dysfunction plays a key role in the pathogenesis of diabetic vascular disease. Activation of nuclear hormone receptor peroxisome proliferator-activated receptor (PPAR)- β and PPAR- γ improved endothelial function in diabetic rats. We studied if the PPAR β agonist GW0742 might exert protective effects in endothelial function in streptozotocin (STZ)-induced diabetic rats.

Methods: The rats were divided into four groups: control, control-treated (GW0742, 5 mg/kg/day by oral gavage), diabetic, and diabetic-treated. Diabetic rats received a single injection via tail vein of STZ 50 mg/kg. GW0742 treatment was followed for 5 weeks.

Results: Long-term GW0742 administration in STZ-rats did not alter plasma glucose, systolic blood pressure and heart rate, but reduced plasma triglyceride levels and increased HDL cholesterol levels. Vasodilatation induced by acetylcholine was decreased and contraction induced by phenylephrine was increased in endothelium-intact aorta from diabetic rats as compared to control. GW0742 restored the endothelial function, without affecting endothelial nitric oxide synthase and caveolin-1 expression in diabetic rats. The aortic superoxide level, the NADPH oxidase

activity, and the mRNA expression for the p22phox, p47phox and NOX-1 subunits of NADPH oxidase were significantly higher in diabetic, but they were lower by GW0742 treatment. Moreover, the up-regulation of prepro ET-1 (ppET-1) found in diabetic rats was also abolished by GW0742.

Conclusion: PPAR β activation improves endothelial function in SZT-diabetic rats. This effect seems to be related to increase in NO bioavailability as a result of reduced NADPH oxidase-driven superoxide production derived to a down-regulation of ppET-1.

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P17-01

ENDOTHELIUM-DEPENDENT VASODILATING AND ANTIOXIDANT PROPERTIES OF A NOVEL ENZYMATIC EXTRACT OF GRAPE POMACE FROM WINE INDUSTRIAL WASTE

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Introduction: Grape pomace (GP) is an industrial waste from the wine process and consists basically of the grape seeds, skins and stems. Its composition rich in phenolic compounds provides to GP promising potential as source of nutraceutical ingredients. GP was extracted by an enzymatic process excluding toxic reagents and high temperatures and pressures. The aim of the present study was to evaluate the effects of that enzymatic extract of GP (GP-EE) on isolated arteries, focusing our attention on its antioxidant properties.

Material and Methods: Vascular effects of GP-EE were evaluated on rat aortic ring and small mesenteric artery (SMA) segments and NO involvement assessed by eNOS inhibition and endothelium removal. ORAC and DPPH assays confirmed antioxidant properties of GP-EE, being vascular superoxide production was assessed by using the fluorescent DHE after incubation with ET-1.

Results: GP-EE (0.0001–0.3 g/l) induced endothelium- and NO-dependent vasodilatation of both vascular beds. In aortic rings but not in SMA, GP-EE (0.01 and 0.03 g/l) was also able to reduce pEC50 (from 7.31 \pm 0.05 to 6.79 \pm 0.09 and 6.69 \pm 0.14) and Emax (from 102.7 \pm 1.98 to 73.88 \pm 2.92 and 58.58 \pm 3.69) of concentration-response curves to Phe. This inhibitory effect was reversed by co-incubation with L-NAME. GP-EE was also able to reduce both O₂⁻ production and contraction elicited by ET-1. GP-EE also prevented vasoconstriction upon inhibition of the antioxidant enzyme SOD with DETCA in aortic rings.

Conclusion: Those results provide evidence that GP is an interesting source of antioxidant compounds and the enzymatic extraction preserves antioxidant and protective vascular properties of grape phenolic compounds.

P18-01

EXPRESSION AND DISTRIBUTION OF PROTEIN KINASE A AND EPAC PROTEINS IN VASCULAR SMOOTH MUSCLE CELLS EFFECT OF CAMP-ELEVATING AGENTS

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Introduction: Cyclic AMP exhibits different patterns of signalling, allowing specific signals to be generated. Here we determine whether caveolae, small invaginations of the plasma membrane rich in cholesterol, interact with the two main cyclic AMP targets (PKA and Epac pro-

teins) in foetal rat aortic smooth muscle cells (A7r5), and the effect of 24 h treatment with cAMP-elevating agents on PKA and Epac expression.

Methods and Results: Caveolin-enriched membrane domains were obtained using a discontinuous sucrose gradient fractionation. The fractions were analysed by SDS-PAGE and western blotting (WB). cav-EPAC and cav-PKA interactions were studied by co-immunoprecipitation. A7r5 express three caveolin isoforms (cav-1, -2 and -3). PKA and caveolin were found in the same light membrane fractions (17–25% sucrose). PKA interacts with cav-1 in control A7r5 cells whereas Epac proteins do not. Protein expression was studied by WB and rt-PCR. A7r5 express PKA-R1I? and both Epac isoforms (1 and 2). PKA expression decreases after 24 h treatment with cAMP-elevating agents (forskolin, an adenylyl cyclase activator); forskolin + IBMX (a non-selective phosphodiesterase inhibitor) or dibutyryl-cAMP (a cAMP analog). At the mRNA level this effect is reproduced by pCPT-cAMP 100 μ M (a PKA activator) and pCPT-2'-O-Me-cAMP 100 μ M (an Epac activator).

Conclusions: These results provide evidence of different compartmentalization of the two main cAMP effectors within vascular myocytes that may contribute to different spatial regulation of cAMP signalling in lipid rafts. Sustained elevation of cyclic AMP decreases PKA expression in the vascular system, therefore become less sensitive to the molecule.

P19-01

ANTIPLATELET EFFECTS OF ALKYL HYDROXYTYROSYL ETHERS IN HUMAN WHOLE BLOOD SAMPLES

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Hydroxytyrosol (HT) is the most abundant polyphenol in olive oil. There is evidence of antithrombotic, antioxidant and neuroprotective HT. These properties are probably responsible for the cardioprotective effects attributed to olive oil. In order to improve the pharmacokinetics/pharmacodynamic profile, several groups of compounds have been developed from hydroxytyrosol, such as alkyl ethers. The aim of this study is to compare the antiplatelet effect of HT with its alkyl ether derivatives. An *in vitro* study was carried out in blood samples from healthy volunteers (N = 6 samples per group). Platelet aggregation was assessed by an electrical impedance, using collagen, arachidonic acid and ADP as inducers. Moreover COX-1 and COX-2 activities, nitric oxide and LPS-induced interleukin-1 beta productions were measured. Seven compounds were tested: acetylsalicylic acid (ASA), hydroxytyrosol (HT), HT ethyl ether (Htet), HT butyl ether, (HT-BTE) HT hexyl ether (HT-HTE), HT octyl ether (HT-OTE) and HT dodecyl ether (HT-DTE). Platelet aggregation was inhibited with all the compounds (ASA: 14, HT: 197, Htet: 79, HT-BTE: 17, HT-HTE: 0.5, HT-OTE: 16, HT-DTE: 48 μ M). COX-1 activity was inhibited in a range into 10(E-4) M. COX-2 activity and interleukin 1-beta were inhibited in a range into 10(E-6)–10(E-5) M. The maximum activity was measured with HT-HTE (1.9 μ M for COX-2) and HT-BTE (3.1 μ M for interleukin).

Conclusions: Alkyl Hydroxytyrosyl ethers inhibit platelet aggregation in human blood samples with a higher potency than HT. A possible effect on the inducible-inflammatory pathways must be deeply studied according to the biochemical results.

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P20-01

ANTIPLATELET EFFECT OF ALKYL HYDROXYTYROSYL ETHERS AFTER ORAL ADMINISTRATION TO RATS

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Hydroxytyrosol (HT) is the most abundant polyphenol in virgin olive oil. There is evidence of antithrombotic, antioxidant and neuroprotective HT properties. These are probably responsible for the cardioprotective effects attributed to olive oil. In order to improve the pharmacokinetics/ pharmacodynamic profile, several groups of compounds have been developed from HT, such as alkyl ethers. The aim of this study is to compare the antiplatelet effect of HT with its alkyl ether derivatives. Six compounds were tested: HT, HT ethyl ether, HT butyl ether, HT hexyl ether, HT octyl ether and HT dodecyl ether. Each compound was administered by gavage in dose of 20 mg/kg/day for 7 days (n = 10 rats per group). Also HT ethyl ether was tested intraperitoneally in dose of 20 mg/kg/day. A control group without treatment was considered. Platelet aggregation was assessed by electrical impedance, using collagen as inducer. Maximum intensity of platelet aggregation (ohms) was inhibited with all the compounds except with HT dodecyl ether (Control: 15.5 \pm 2.0, HT: 14.3 \pm 0.8, HT-ethyl ether: 12.0 \pm 1.6, HT-butyl ether: 6.4 \pm 0.7, HT-hexyl ether: 6.5 \pm 0.8, HT-octyl ether: 10.2 \pm 1.7, HT-dodecyl ether: 15.7 \pm 2.3). Intraperitoneal administration of HT-ethyl ether also inhibited platelet aggregation (7.0 \pm 0.4 vs. 12.0 \pm 1.6 ohms in the oral administration group and 15.5 \pm 2.0 in control group).

Conclusions: Oral administration of alkyl hydroxytyrosyl ethers inhibited whole blood platelet aggregation in rats mainly butyl and hexyl derivatives.

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P21-01

BLOOD OXIDATIVE STRESS AND PLATELET PARAMETERS IN PEDIATRIC PATIENTS UNDERGOING HEART SURGERY

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Despite the new analytical methods and therapeutic management of post-operative pediatric cardiovascular surgery, there is still an issue not completely clear, the real importance of oxidative stress generated during ischemia-reperfusion after cardiopulmonary bypass. Systematically analyze the correlation between oxidative stress and various clinical and laboratory parameters in postoperative cardiac surgery using cardiopulmonary bypass in children younger than 15 years. In this study we show the results for a pilot study to assess changes in oxidative stress and platelet function caused by heart surgery. This pilot study was carried out in 30 patients. Each patient underwent serum measurements on the same day of surgery, one before it, then coming out of cardiopulmonary bypass, and 18–24 h of their arrival in the pediatric intensive care. In each sample is then analyzed and determination of blood oxidative stress parameters and platelet aggregation. Plasma levels of glutathione: Pre-Q 3.233 \pm 0.782, 2.329 \pm Post-Q 0.691, 2.992 \pm 18 h post-0.711 (nmol/mg protein), plasma MDA levels: Pre-Q 0.1504 \pm 0.0473, Post-0.2163 \pm 0.0912 Q, Post-18 h \pm 0.1895 0.0692 (nmol/mg protein). Collagen-induced aggregation: Pre-Q 28.60 \pm 4.22, Post-Q 15.20 \pm 5.07, Post-18 h 20.60 \pm 3.98 (ohm) with arachidonic acid: Pre-Q 25, 00 \pm 2.94, Post-Q 14.10 \pm 1.52, Post-18 h 19.50 \pm 1.43 (ohm), with ADP: Pre-Q 22.90 \pm 1.66, Post-Q 12, 90 \pm 1.52, 18.00 \pm 18 h post-2.49 (ohm).

Conclusion: There is an oxidative stress during reperfusion and activation of platelet function during surgery.

P22-01

RESVERATROL INDUCES NUCLEAR FACTOR-KB ACTIVITY IN HUMAN CARDIAC CELLS TO EXERT ITS CARDIOPROTECTIVE EFFECTS

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Introduction: Resveratrol is a polyphenolic compound present in grape that prevents cardiac hypertrophy and protects the heart from ischemic injury, metabolic dysregulation, and inflammatory processes in several murine models. However, little is known about its effects on human cardiac cells.

Material and Methods: Cardiac cells of human origin (AC16) or rat neonatal cardiomyocytes were treated with resveratrol (30–50 μ M) and TNF- α (100 ng/ml). Mice were fed a standard chow diet or a standard chow diet supplemented with resveratrol (1 g/kg diet) for 4 months.

Results: TNF- α induced the DNA-binding activity of the pro-inflammatory transcription factor NF- κ B. Unexpectedly, addition of resveratrol, alone or in combination with TNF- α , induced even more the NF- κ B transcriptional activity. In accordance with this, resveratrol also increased the expression of the pro-inflammatory genes *ICAM-1* and *TNF- α* . Likewise, resveratrol also induced inflammatory processes in murine cardiac cells. Conversely, resveratrol displayed an anti-inflammatory effect with regard to *IL-6* and *MCP-1* expression, regardless the presence of TNF- α . Western-blot analyses revealed that NF- κ B p65 subunit levels were up-regulated in an I κ B-dependent manner in nuclei of human cardiac cells of resveratrol-treated cells. Finally, we demonstrate that resveratrol activates the STAT3 signalling pathway and induces the expression of anti-apoptotic Bcl-xL, both related with the cardioprotective SAFE (survival activating factor enhancement) pathway.

Conclusion: Resveratrol enhances NF- κ B activity in human and murine cardiac cells, in a process that coincides with the activation of STAT3 and anti-apoptotic downstream effectors. Therefore, activation of the SAFE pathway by resveratrol might be involved in the cardioprotective effects of this compound.

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PAIN AND INFLAMMATION

P23-02

ROLE OF HO-1 IN THE EFFECTOR PHASE OF ARTHRITIS

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Introduction: Rheumatoid arthritis is an autoimmune systemic disease that mainly affects the synovial cavity of joints and produces a progressive destruction of cartilage and bone. Several studies have reported cytoprotective effects of heme-oxygenase (HO), which removes potentially toxic pro-oxidant molecules and increases the amount of antioxidants of heme metabolism. The aim of this study was to evaluate the involvement of HO-1 in the effector phase of arthritis.

Material and Methods: We used an animal model that reproduces the initial acute phase of the inflammatory response of human arthritis, the K/BxN serum transfer model. We evaluated the development of the disease in HO-1 Knock-out (-/-) (KO), heterozygous (+/-) (HT) and their corresponding wild type (+/+) (WT) mice by macroscopic and histological studies and determined the levels of diverse mediators by ELISA and RIA techniques.

Results: HT and KO mice showed a worsening in the development of arthritis (macroscopic and histological score). Among the studied pro-inflammatory mediators, we saw a significant increase in TNF- α , MMP-3, IL-6 and PGE₂ in ankles homogenate and serum of HT and KO mice respect to arthritic WT. Moreover, the levels of anti-inflammatory cytokines such as IL-10 and IFN- γ were reduced in HT and KO mice.

Conclusion: These data demonstrate that HO-1 enzyme plays an anti-inflammatory role and protects against the joint damage associated with arthritis. Therefore, understanding the mechanism by which HO-1 activation confers protection against inflammatory processes may be a useful tool for developing preventive and therapeutic strategies for treating the inflammation associated with autoimmune disorders.

P24-02

INVOLVEMENT OF TETRODOTOXIN-SENSITIVE SODIUM CHANNELS IN VISCERAL PAIN

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Introduction: Visceral pain is the most common form of pain produced by disease, and one of the most frequent reasons why patients seek medical attention. Despite its importance and prevalence the mechanisms involved are still poorly understood. There is strong evidence for the involvement of voltage-gated sodium channels (Na_v) in pain transmission. The role of Na_v1.7, a tetrodotoxin sensitive channel (TTX-S), in the somatic pain is well established and some authors suggest its involvement in visceral pain. In spite of the importance of Na_v1.7 in pain transmission, there are few data that investigate the participation of TTX-S in visceral pain.

Material and Methods: Wild-type (WT) and conditional knockout (KOC) mice in which Na_v1.7 was removed in nociceptive DRG neurons were used. These mice were evaluated in models of inflammatory (intracolonic instillation of mustard oil) and not inflammatory (intracolonic instillation of capsaicin) visceral pain. We evaluated behavioral responses and subsequent referred hyperalgesia by Von Frey filaments. TTX s.c. was used (1–3 μ g/kg capsaicin, 3–6 μ g/kg in mustard oil) to block TTX-S channels, including Na_v1.7.

Results: We did not observe differences between WT and Na_v1.7 KOC mice in any of the models evaluated. However, animals treated s.c. with TTX showed a statistically significant decrease in the number of pain responses.

Conclusion: These results indicate that Na_v1.7 is not involved in visceral pain models and also suggest the important role of others TTX-S channels.

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P25-02

5-HT1A RECEPTOR'S ROLE IN THE ANALGESIC EFFECT OF M-OPIOIDS AGONIST IN THE RAT FOREBRAIN

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Background: The analgesic effects of μ -opioids agonist can be blocked by selective 5-HT1A antagonist. 5-HT1A selective agonist and antidepressants able to up-regulate the brain 5-HT1A have shown analgesic effect.

Aim: To characterized the possible relationship between μ -opioids acute stimulation and rat forebrain 5-HT1A receptors.

Method: Male Wistar rats (n = 48, 6 months old, 345 \pm 15 g) were injected intraperitoneally with single dose of Fentanyl 3.2, 6.6 or 12.8 μ g/kg vs. saline in absence and presence of the 5-HT1A selective antagonist WAY 100635 (0.5 mg/kg). Fentanyl analgesia was assessed with nociceptive mechanical stimulation (Randall-Selitto testing), 30 min after drug injection. Serotonin 5-HT1A receptors in the rat forebrain were characterized using autoradiography techniques with 3H-8OH-DPAT.

Results: Fentanyl 12.8 μ g/kg increased the 5-HT1A receptors maximal bound in (control vs. Fentanyl): hypothalamus (15.5 \pm 0.98 vs. 35.3 \pm 4.98 fmol/mg protein, +127%), frontoparietal areas (56.6 \pm 8.79 vs. 91.9 \pm 10.6 fmol/mg protein, +113%), hippocampus CA1 (73.8 \pm 2.93 vs. 156 \pm 5.16 fmol/mg protein, +111%) and DGm (80.3 \pm 5.34 vs. 128 \pm 6.54 fmol/mg protein, +60%), amygdalin nuclei PMCo (55.03 \pm 6.57 vs. 110.9 \pm 22.2 fmol/mg protein, +101%) and AHial (32.2 \pm 2.4 vs. 61.8 \pm 4.77 fmol/mg protein, +92%). Both the analgesia response and the 5-HT1A receptors maximal bound increasing were attenuated by WAY 100635. The 5-HT1A receptors up-regulation induced by Fentanyl may be implying in the synergistic effects described for μ -opioids agonists and specific serotonergic anti-depressants in acute and chronic pain treatment.

Conclusion: μ -opioids agonist up-regulated serotonin 5-HT1A receptors in the rat forebrain.

P26-02

ELLAGIC ACID, A NATURAL DIETARY POLYPHENOL, SUPPRESSES COX-2 EXPRESSION IN HUMAN MONOCYTE THP-1/MACROPHAGES THROUGH MAPK SIGNALLING PATHWAY

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Background: Monocytes and macrophages play a key role in the immunopathogenesis of inflammatory based diseases. Ellagic Acid (EA), a natural polyphenol compound present in certain fruits such as berries and pomegranate, has recently been subject of intense research within cancer and inflammation fields due to its multiple pharmacological actions.

Aims: The present study was designed to investigate the anti-inflammatory and antioxidant role of EA in LPS-stimulated THP-1 monocytes and PMA-differentiated macrophages. Given that cyclooxygenase (COX)-2 is upregulated by mitogen-activated protein kinases (MAPKs) and NF- κ B

nuclear transcription factor, we determined the effects of EA treatment in COX-2 expression and the involvement of p38, JNK and ERK1/2 MAPKs and NF- κ B signalling pathways.

Methods: Cell viability was assayed by MTT and SRB assay in monocytes and macrophages, respectively. The intracellular reactive oxygen species (ROS) formation was measured using 2,7-dichlorofluorescein diacetate (DCFHDA) fluorescent probe and DPPH free radical scavenging assay. Changes in COX-2, MAPKs and inhibitory protein of NF- κ B (I κ B α) expressions after EA treatment were detected by western blotting.

Results: EA treatment did not produce any changes in cell viability. However, EA-treated cells (10, 25 and 50 μ M) reduced ROS generation in monocytes and macrophages ($P < 0.01$) and induced a significant COX-2 downregulation ($P < 0.01$). Besides, EA-treated monocytes and macrophages showed significant decreases in MAPKs protein phosphorylation ($P < 0.05$) whereas no significant changes in I κ B- α protein expression were observed after EA treatment.

Conclusion: These novel findings suggest that EA could exert a protective/preventive role in inflammatory based diseases probably through MAPKs signalling pathway.

P27-02

DIETARY POMEGRANATE EXTRACT AMELIORATES CHRONIC COLITIS. INVOLVEMENT OF MITOGEN ACTIVATED PROTEIN KINASE PATHWAYS

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Introduction: *In vitro* and *in vivo* studies have demonstrated antioxidant and anti-inflammatory properties of pomegranate, (*Punica granatum* L). Inflammatory bowel disease is characterized by the development of an abnormal immune and inflammatory response. A complex system of intracellular signaling molecules such as mitogen-activated protein kinases (MAPKs) influences this uncontrolled immune system activation and inflammation by ultimately modulating gene transcription.

Material and Methods: To gain a better understanding of the effects and mechanisms of action of a validated pomegranate extract (PE, 250 and 500 mg/kg), on the chronic injury caused by intra-colonic administration of trinitrobenzenesulfonic acid (TNBS) in rats, 4 weeks old-Wistar male rats were randomized into three dietary groups: (i) standard diet, (ii) pomegranate extract (PE) 0.6% and (iii) PE 1.1% diets. Rats were fed with diets during 30 days before TNBS instillation and during 2 weeks before killing. The inflammatory response was assessed by gross appearance, myeloperoxidase activity (MPO), and TNF- α production. Also, pro-inflammatory proteins (COX-2 and iNOS) and c-Jun N-terminal kinase (JNK) and p38 MAPKs, were studied by western blotting.

Results: Dietary PE significantly ($P < 0.001$) reduced the colonic damage. In addition, the degree of neutrophil infiltration as well as TNF- α levels were significantly ameliorated after the intake of PE-enriched diets. A higher COX-2 and iNOS protein expression were detected in colon mucosa from control TNBS treated rats. On the contrary, dietary PE ingestion drastically decreased both proteins overexpression and reduced the activation of p38 and JNK MAPKs.

Conclusion: These data suggest that PE-enriched diets consumption may have a therapeutic role in chronic ulcerative colitis reducing the oxidative events and returning pro-inflammatory proteins expression to basal levels probably through MAPKs signaling pathway.

P28-02

A NEW SIGNALING PATHWAY THROUGH IRAK-M INVOLVED IN THE ALPHA-7 NICOTINIC RECEPTOR-MEDIATED ANTI-INFLAMMATORY RESPONSE IN HUMAN MACROPHAGES

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The interleukin-1 receptor-associated kinase-M (IRAK-M) is primarily expressed in monocytes and macrophages and functions as a negative regulator of Toll-like receptor signaling. Given that activation of alpha7 nicotinic acetylcholine receptor of macrophages mitigates inflammation, we have investigated whether this alpha7-mediated effect is related to the IRAK-M expression and/or phagocytic activity in these immune cells. Human macrophages and the mouse macrophage cell line RAW 264.7, and a set of experimental approaches, including real time quantitative PCR, western blot, confocal microscopy and flow cytometry, were used throughout this study. Exposure of macrophages to nicotine (1 nM–100 μ M; from 6 to 72 h) increases phagocytosis and expression of IRAK-M mRNA and protein in a concentration- and time-dependent manner. Both effects are due to the activation of alpha7 receptors expressed by these cells since they are completely blocked by alpha-bungarotoxin (1 μ M) and significantly enhanced by PNU120596 (1–30 μ M), a selective blocker and a positive allosteric modulator of alpha7 receptors, respectively. The signaling pathway connecting alpha7 with IRAK-M was analyzed by using selective inhibitors of different kinases (MEK, MAPK ERK 1/2, MAPK p-38, JAK-2, PI3K) and transcription factors (AP1, STAT3). Only AG490 (10 μ M), STA-21 (20 μ M) and LY-294002 (1 μ M) reduced significantly (above 50%) the up-regulation of IRAK-M elicited by nicotine (100 nM). Thus, our results suggest a role for JAK2-STAT3 and PI3K activation in the alpha7-mediated up-regulation of IRAK-M, and reveal a new mechanism implicated in the cholinergic anti-inflammatory response in human macrophages. Supported by Ministerio de Ciencia e Innovación (SAF2008-05347) and Fundación Mutua Madrileña

P29-02

THE SPECIFICITY AND MAGNITUDE OF THE IMMUNE RESPONSE RAISED AGAINST FOOD ALLERGENS DEPENDS ON THE MURINE EXPERIMENTAL MODEL USED

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Introduction: Food allergies occur in 5–10% of children until 2 years of age. The most common is to cow milk proteins (CMP), an allergy that affects between 2 and 6% of children under 2 years of age. The importance of this pathology is neither due to the high incidence nor to the symptoms induced, but because it triggers the so-called 'atopic career', which has been associated with an increase in the risk to acquire other diseases such as asthma, rhinitis and atopic dermatitis in adult age. The possible mechanisms are multiple, but probably factors such as an altered immune response during neonatal states could be a key process.

Material and Methods: To evaluate the immune response exerted by some food allergens we tested several murine experimental models of allergy analyzing the incidence of the allergen (Ovalbumin vs. cow's milk protein), route of sensitization and challenge (oral vs. I.P.), duration

and also purity and nature of the antigen. In all these allergy models, clinical score, histamine levels, and colon and plasma cytokines and immunoglobulin levels were analyzed.

Results and Conclusions: In general, I.P. challenge and long sensitization protocols exert a higher immune response measured as an increased clinical score and Th2 cytokines such as IL-5 and Ig levels, especially IgG1 and IgE. Moreover, I.P. challenge and the purity/nature of the antigen are related to a more specific immune response, since a general increase on the immune response was not observed in allergic animals but only in response to the allergen.

P30-02

PERIBULBAR ANAESTHESIA PROVIDED MORE EFFECTIVE AND SAFER POST-SURGICAL ANALGESIA THAN RETROBULBAR BLOCK AND GENERAL ANAESTHESIA IN CATARACT SURGERY

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Introduction and Aims: Cataract surgery is a frequent surgical procedure requiring anaesthesia.

Aims: To compare the efficacy and safety of general vs. peribulbar vs. retrobulbar anaesthesia in cataract surgery. To determine the postoperative rescue analgesia requirement after these three types of anaesthesia.

Method: A multicentre, prospective, transversal, descriptive, analyzer single-blind trial was done in patient undergoing cataract surgery. ASA I-III patients were subjected to general (GA), peribulbar (PB) or retrobulbar (RB) anaesthesia. Epidemiologic, clinical and analytical data, type of surgery, type of anaesthesia, perioperative events, intraocular pressure, ocular akinesia, post-surgery pain intensity, postoperative rescue analgesia requirement, incidence and type of early and late adverse reactions. The primary outcomes were pain intensity and number of patients requiring rescue analgesia during the 24-h study period. Secondary outcomes assessed were sedation scores, ocular akinesia, time to first rescue analgesia, nausea/vomiting, patient acceptability and ocular and systemic complications.

Results: One hundred and eighty-seven patients 69.6 ± 25 years, 51.3% males, underwent GA (N = 66), RB (N = 62) or PB (N = 59), cataract surgery. Peribulbar block was statistically superior (P < 0.05) to RB and GA (PB/RB/GA): number of patients required rescue analgesia during 24 h-follow up were 28.8%/35.4%/37.87%; mean postoperative pain scores were 4.5/5.2/6.1 at 6 h post-surgery; the median time to first rescue analgesia were 8.4/6.2/5.4. Sedation rate was GA>RB=PB; perioperative discomfort was GA>>RB>PB. GA was related with nausea/vomiting 22.7% and hypotension 7.5%; No complication related to PB was noticed.

Conclusion: Peribulbar anaesthesia provided more effective and safer postsurgical analgesia than retrobulbar block and general anaesthesia in cataract surgery.

P31-02

THE SPECIFICITY AND MAGNITUDE OF THE IMMUNE RESPONSE RAISED AGAINST FOOD ALLERGENS DEPENDS ON THE MURINE EXPERIMENTAL MODEL USED

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Introduction: Food allergies have been associated with an increase in the risk to acquire other immune-related diseases. The possible mechanisms involved are multiple, but probably factors such as increased permeability in intestinal barrier during neonatal states and the immature development of immune system are key processes. In addition, local alteration produced during atopic response could determine or modify the innate immune system activation against luminal antigens, thus altering oral tolerance induction and consequently affecting immune system maturation.

Material and Methods: To test this hypothesis, we have evaluated the severity and the immune response involved in two intestinal inflammatory models (DSS and DNFB/DNS) in mice that have suffered or not a previous episode of allergy. First, the cow's milk allergy was induced in 3 week-old Balb/c mice followed by a DSS or DNFB/DNS IBD model 2 weeks latter of allergy recovery (at 8–10 week-old).

Results: Although no major differences were observed in the clinical score nor in the macroscopically evaluation of the intestinal inflammation in the atopic animals compared to the control ones, at the immunological level several differences could be observed between both groups involving for example the number of intestinal patches or the levels of IgE and IgG1 both in plasma and colon. These differences were higher in those animals with a bigger severity of the intestinal inflammatory process.

Conclusions: The differences in the immune response observed in atopic animals could be involved on the increase risk to acquire other immune-related diseases in allergic individuals.

P32-02

USE PATTERN OF OPIOID ANALGESICS AT THREE DIFFERENT GEOGRAPHICAL LEVELS IN SPAIN, 2006–2008

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Introduction: Opioid analgesic medication is the mainstay for severe pain management. The objective was to describe trends in opioid consumption in Spain, the region of Castilla y León, and the province of León over 3 years (2006–2008).

Material and Methods: Data for the period studied were drawn from the ECOM and Concyliya databases (Spanish Ministry of Health and Regional Service of Health, respectively), and converted into Defined Daily Dose (DDD)/1000 inhabitants per day and cost per day.

Results and Conclusions: Consumption of opioids increased by 36–41% depending on the area considered (from 6.545 in 2006 to 8.932 DDD/1000 inhabitants per day in 2008 in Spain; 4.578 to 6.211 DDD/1000 inhabitants per day in Castilla y León; 4.699 to 6.621 DDD/1000 inhabitants per day in León). The most consumed drugs in terms of DDD/1000 inhabitants per day were tramadol followed by fentanyl. Considering costs per day, the highest increase has been observed in data corresponding to León (9.49 € a day in 2006 to 14.20 € in 2008, rise of 50%), whereas in Spain and Castilla y León, the increase is below 40% (Spain, from 11.69 to 15.58 €; Castilla y León, 9.68 to 13.40 €). Fentanyl is always the most expensive drug, accounting for nearly half the cost per day of the group. A similar trend in opioid use has been observed in these three geographical areas, with an important increase in opioid consumption, and with tramadol and fentanyl as the most prescribed drugs.

P33-02**INDUCTION OF GENES IN THE NOTCH AND WNT SIGNALING PATHWAYS BY HYPOXIA IN MACROPHAGES AND GASTROINTESTINAL EPITHELIAL CELLS**

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Release of mediators from inflammatory cells may modulate the ability of epithelial cells to regenerate damaged areas in the inflammatory bowel disease (IBD). In epithelial cells, hypoxia has been shown to promote epithelial barrier function through HIF-dependent transcriptional regulation. Wnt and Notch signaling pathways are involved in proliferation of intestinal crypt cells and secretory cell differentiation.

Aim: To analyse effects of hypoxia on the expression of Wnt and Notch ligands and receptors in macrophages and epithelial cells.

Material and Methods: Human macrophages (U937) and epithelial cells (AGS and Caco-2) were exposed to normoxia or hypoxia for different times (0, 3, 5, 8, 16 and 24 h). HIF-1 α and HIF-2 α protein levels were analyzed by Western blot. Gene expression of Wnt ligands (Wnt5a, Wnt3a and Wnt1), receptors (Fzd1 and Fzd5) and a target gene (Lgr5) as well as Notch ligands (Jag1 and Dll4) and receptors (Notch1, Notch3 and Notch4) was analyzed by real-time RT-PCR.

Results: Hypoxia induced a time-dependent increase in Jag1 and Wnt1 mRNA expression in macrophages that peaked at 5 or 16 h, respectively. In AGS hypoxia increased Fzd5, Notch1 and Notch4 receptors and Lgr5 expression which peaked at 24, 3 and 3 and 5 h, respectively. In Caco-2 hypoxia increase Fzd5 and Notch4 expression at 5 h. HIF-1 α and HIF-2 α stabilization was induced by hypoxia in all cells analyzed.

Conclusion: Hypoxia can modulate mechanisms of epithelial proliferation and differentiation at IBD through the expression of several Wnt and Notch ligands in macrophages and up-regulation of its receptors in epithelial cells.

P34-02**LABDANE DITERPENES EXERTS ANTI-INFLAMMATORY EFFECTS AND INCREASES SURVIVAL IN ENDOTOXEMIC MICE**

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Introduction: Terpenoids are considered very promising starting points for the development of new therapeutic agents. These compounds have been widely used in the treatment of infection and inflammatory diseases. In this context, a series of labdane diterpenes derivatives (1–9) were prepared from labdaneidol and their anti-inflammatory potential was evaluated on lipopolysaccharide (LPS)-induced inflammatory responses. The signaling pathways involved in the anti-inflammatory effects of diterpenes and the therapeutic effect in a mouse sepsis model were also evaluated.

Material and Methods: Macrophages were cultured and activated with LPS in the absence and presence of diterpenes. Inhibitory effects of the compounds on nitric oxide (NO), prostaglandin E2 (PGE2) and inflammatory cytokines release were evaluated. Involvement of NF- κ B and MAPK signaling was determined by Western blot and confocal microscopy. Protective effects in a sepsis model in mice were elucidated by determining survival and circulatory IL-6 and TNF- α concentrations.

Results: From these compounds, labdane 5 (labdanoic acid methyl ester: LAME) showed the most potent anti-inflammatory effect due to the reduction of NOS-2 and COX-2 gene expression, acting at the transcriptional level. Inflammatory cytokines (IL-6, TNF- α and IP-10) were downregulated in the presence of LAME. These effects are mediated by

inhibition of MAPK activation and nuclear translocation of NF- κ B. Additionally, LAME improved survival in a mouse model of endotoxemia, reducing the circulatory levels of cytokines.

Conclusions: LAME significantly attenuates the pro-inflammatory response induced by LPS both *in vitro* and *in vivo*.

P35-02**VARIATION IN SIRT1 EXPRESSION IN KO IL10-/- MICE THAT EVOLVED FROM CHRONIC INTESTINAL INFLAMMATION TO COLON CANCER**

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Introduction: Interleukin-10-deficient (IL-10 KO) mice develop colitis and colorectal cancer similar to the inflammatory bowel disease (IBD) associated cancer in humans. Therefore, these animals are considered excellent models for identifying the complex mechanisms involved in different types of human IBD. Sirtuins are histone deacetylases (HDACs) type III. They are widely distributed in living organisms, from yeast to mammals which have been described up to seven different types. In recent years, it has been shown to regulate a variety of pathophysiological processes, including inflammation, cellular senescence, apoptosis, differentiation and cancer-related processes and metabolism.

Material and Methods: C57BL/6 mouse IL10-KO and wild-type (WT) were used. Animals were maintained under specific-pathogen free conditions and transferred to conventional housing at aged 4 weeks. After a period of adaptation of 2 weeks, the study started. Animals from both groups were sacrificed at 0, 4, 8 and 12 weeks after the experiment was initiated. Expression of Sirt1 and TNF- α (mRNA) was analyzed by qRT-PCR. The study was supplemented by histological and immunohistochemical analysis.

Results: IL10-/- animals presented lower levels of Sirt1 to control animals as well as higher levels of TNF- α , as expected after macroscopic and immunohistochemical observations of each of the samples.

Conclusion: The study model used allows us to continue studying the implications of sirtuins in the development of inflammation and cancer. We found that the enzyme levels are decreased, while levels of TNF- α increase. We are interested in studying the degree of expression of other genes involved in processes of inflammation and cancer.

P36-02**DERMAL FIBROBLASTS FROM PSORIATIC PATIENTS ELICIT REDUCED COX-2 INDUCTION AND PROSTAGLANDIN E2 (PGE2) PRODUCTION**

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Introduction: Dermal fibroblasts could contribute to psoriasis pathogenesis by producing soluble mediators involved in T-cell recruitment and activation. In particular, fibroblast-derived prostaglandin E2 (PGE2) induces dendritic cell release of IL-23. However, non-steroidal anti-inflammatory agents cause disease exacerbation, suggesting a protective role for prostanoids.

Material and Methods: Fibroblasts were isolated from foreskin of nine healthy donors or from lesional skin biopsies of 12 psoriatic patients. After 24 h stimulation with TNF- α (0, 1, 2.5, 10 or 25 ng/ml) or the protein kinase C (PKC) activator 12-O-tetradecanoylphorbol-13-acetate (TPA, 1 μ g/ml), supernatants PGE2 levels were determined by RIA. COX-2 expression was detected by immunocytochemistry or western blot of whole cell lysates, using IL-1 β (2.5 ng/ml) as positive control.

IL-6 and IL-8 were detected by ELISA. Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction.

Results: In healthy fibroblasts, both TNF- α (10 ng/ml) and TPA similarly induced PGE2 production (7.56 ± 1.38 and 8.13 ± 1.38 ng/ml, respectively vs. untreated 1.77 ± 0.51 ng/ml, $P < 0.001$). In contrast, fibroblasts from psoriatic lesions failed to produce significant levels of PGE2 upon TNF- α or TPA stimulation (0.55 ± 0.18 and 0.70 ± 0.25 ng/ml, respectively vs. untreated 0.29 ± 0.09 ng/ml, $P = \text{NS}$), which correlated with diminished induction of COX-2. We further observed a diminished IL-6 and, to a lesser extent, IL-8 secretion by stimulated psoriatic fibroblasts.

Conclusion: Our findings support the view that fibroblast phenotype is altered by chronic inflammation, as occurs in psoriasis.

P37-02

NEW INHIBITORS OF IL-6 PRODUCTION IN CACO-2 CELLS THROUGH MOLECULAR TOPOLOGY METHODOLOGY

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Introduction: A type of quantitative structure-activity relationship (QSAR) methodology, Molecular topology, a formalism based on describing the molecules with the aim of topological descriptors, has been used to the search of new active compounds against Inflammatory bowel disease (IBD). Interleukin (IL)-6, a pro-inflammatory cytokine, plays a key part in the uncontrolled intestinal inflammatory process, which is a main characteristic of IBD.

Material and Methods: Based in a Virtual Screening carried out by our research group and previously published, it was selected a group of 28 compounds with potentially predicted activity vs. UC. Four of them, named: Alizarin -3-methylindioacetic acid, Calcein, (+)-Dibenzyl-L-tartrate and Ro 41-0960, were finally tested *in vitro* at 10, 50 and 100 μM . Cytotoxicity of compounds was tested by the MTT assay. CACO-2 cells were treated with IL-1 β (25 ng/ml), TNF- α (50 ng/ml) and IFN- γ (50 ng/ml), and then it was evaluated IL-6 levels on supernatants by ELISA.

Results and Conclusion: All compounds show at least a cell viability percentage of 70% at the highest tested concentration. (+)-Dibenzyl-L-tartrate and Calcein (100 μM) and Ro 41-0960 (50 μM) were able to significantly inhibit IL-6 production by at least 83%. This result confirm the efficiency of Molecular Topology's strategy for the selection of compounds potentially active in ulcerative colitis.

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Reference:

1. Gálvez-Llompert et al. (2011) Mol Divers. DOI: 10.1007/s11030-011-9323-4.

P38-02

CHRONIC TREATMENT WITH NALTREXONE INDUCES NEUROCHEMICAL AND FUNCTIONAL SUPERSENSITIVITY TO SUFENTANIL BY PROMOTING M-OPIOID RECEPTOR COUPLING TO G α_z PROTEINS

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Introduction: Opioid receptors are coupled to pertussis toxin (PTX)-sensitive, heterotrimeric G α_i /G α_o proteins. Sustained administration of

opioid antagonists results in an enhanced antinociceptive response to agonists. We investigated the changes in spinal cord μ -opioid receptor signalling underlying this phenomenon.

Material and Methods: Rats received naltrexone (120 $\mu\text{g/h}$; 7 days) via osmotic minipumps. The antinociceptive response to the μ -agonist sufentanil was tested 24 h after naltrexone withdrawal. The interaction of μ -receptors with G α proteins (agonist-stimulated [³⁵S]GTP γ S binding and immunoprecipitation of [³⁵S]GTP γ S-labelled G α subunits) and μ -receptor-dependent inhibition of the adenylyl cyclase (AC) activity were determined.

Results: Chronic naltrexone treatment augmented DAMGO-stimulated [³⁵S]GTP γ S binding, potentiated the inhibitory effect of DAMGO on the AC/cAMP pathway, and increased the inverse agonist effect of naltrexone on cAMP accumulation. In control rats, the inhibitory effect of DAMGO on cAMP production was antagonized by pertussis toxin (PTX) whereas, after chronic naltrexone, the effect became resistant to the toxin, suggesting a coupling of μ -receptors to PTX-insensitive G α_z subunits. Immunoprecipitation assays confirmed the transduction switch from G $\alpha_{i/o}$ to G α_z proteins. The consequence was an enhancement of the antinociceptive response to sufentanil that, in consonance with the neurochemical data, was prevented by G α_z antisense oligodeoxyribonucleotides but not by PTX.

Conclusion: Following chronic naltrexone, μ -opioid receptors in the spinal cord experience a transduction shift from PTX-sensitive G $\alpha_{i/o}$ to PTX-resistant G α_z proteins. Consequently, the inhibitory effect of DAMGO on the AC/cAMP pathway is enhanced. This transduction change is responsible for an increased pharmacological response to agonists.

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P39-02

DISCOVERING NOVEL ANTI-INFLAMMATORY DRUG-LIKE COMPOUNDS BY ALIGNING IN SILICO AND IN VIVO SCREENING: THE NITRO-INDAZOLINONE CHEMOTYPE

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The combination of computational methods and *in vivo* screening is proposed as a novel strategy to anti-inflammatory drug discovery. The TOMOCOMD-CARDD descriptors and linear discriminant analysis are used to develop a total of 13 models. The best model (Equation 13) shows an accuracy of 87.70% and 88.44% in the training and test sets, respectively. The equation is used for the identification of anti-inflammatory compounds using *virtual screening* of 145 molecules available in our *in-house* library. Out of these, 34 chemicals were selected and tested in a new *in vivo* anti-inflammatory test using *Danio rerio* (zebrafish) larvae. This activity was evaluated following the leukocyte migration to damaged zone (end tail). Seven compounds gave the best results with 65–84% of anti-inflammatory activity. We also evaluated the most potent compounds in an *in vivo* test by using phorbol 12-myristate 13-acetate-induced mouse ear oedema. Four compounds showed similar values to indomethacin. Compound 12 (VA5-131, 2-benzyl-1-methyl-5-nitro-1,2-dihydroindazol-3-one) was the most active and completely abolished the oedema. Evidently, this study suggests a new support structure (nitro-indazolinone chemotype) that constitutes a novel promising lead and may represent an important therapeutic alternative for the treatment of inflammatory conditions.

NEUROPSYCHOPHARMACOLOGY

P40-03

ROLE OF HO-1 IN THE EFFECTOR PHASE OF ARTHRITIS FINASTERIDE AND ALLO-PREGNENOLONE ADMINISTRATION ON THE ANXIOLYTIC EFFECT OF MUSIC IN FEMALE RATS. INTRODUCTION

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Previous studies in rodents show that ovarian hormones, specially progesterone (PG), are involved in the anxiolytic effect of music. Mainly, this research assessed the influence of PG derivatives. For this purpose two experiments were designed: (i) Allo-pregnenolone (alloP) administration to ovariectomized rats and (ii) Administration of finasteride, 5 α reductase inhibitor, to intact female rats. Both models are subjected to silence, noise and music (Mozart Sonate K448). Anxiety was evaluated by elevated plus maze (EPM) for 5 min, estimating time spent in open arm (TOA) and total number entries (TNE). Experiment 1 was conducted during 14 days after ovariectomy, being alloP injected 2 h before measuring TOA and TNE. Similarly, 2 h before of assay, finasteride was injected. Ovariectomy significantly decreased TOA and TNE in silence and in lesser degree after music. Finasteride prompted a significant decreases in three experimental conditions ($P < 0.001$). The data suggest that PG, mainly alloP, play an important role in anxiolytic effect of music. This effect may be due, at least, to ambient created by music in presence of PG derivatives. In the other hand, it is well demonstrated the interactions between these compounds and GABA-receptors, and their anxiolytic action.

P41-03

CANNABINOID CB₁ RECEPTOR PROTEIN IS DOWN-REGULATED IN THE CEREBRAL CORTEX OF COCAINE ADDICTS AND COCAINE-TREATED RATS

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Endocannabinoids and CB₁ receptors are involved in the effects of drugs of abuse like cocaine. Chronic exposure to cocaine was shown to decrease the expression of CB₁ receptor mRNA without altering the number of agonist (³H-CP-55,940) binding sites in rat cerebral cortex. The aims of this study were to investigate CB₁ receptor regulation in brains of cocaine addicts and cocaine-treated rats. Postmortem prefrontal cortices (PFC/BA9) were collected from nine cocaine addicts (5M/4F; 35 \pm 4 years; 28 \pm 7 PMD) and nine healthy matched controls for each group. Long-term drug abuse was revealed by cocaine in blood and hair samples. Male Sprague-Dawley rats were exposed (i.p.) to saline ($n = 5$) or cocaine (acute: 20 mg/kg, 1 h, $n = 6$; chronic: 40 mg/kg, 6 days, $n = 5$; chronic plus withdrawal: 3 days, $n = 5$). CB₁ receptor protein was detected by Western. The immunodensity of CB₁ receptor protein (PFC/BA9) was reduced in cocaine addicts (55 \pm 11%, $P = 0.001$) when compared with that in sex-, age-, and PMD-matched controls. Chronic cocaine (and cocaine abstinence), but not acute cocaine, was associated with a marked downregulation of CB₁ receptors in rat cerebral cortex (75–80%, $P < 0.0001$). Chronic exposure to cocaine is associated with reduced CB₁ receptor protein in brain, indicating a role of the endocannabinoid system in the mechanisms of dependence for cocaine in the PFC/BA of human addicts. The downregulation of CB₁ receptor protein induced by cocaine may be related to sustained receptor stimulation mediated by the endocannabinoid agonist anandamide.

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P42-03

GLUCOCORTICOID RECEPTOR INVOLVEMENT IN NORADRENERGIC HYPERACTIVITY DURING MORPHINE WITHDRAWAL

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Noradrenergic system and the hypothalamus-pituitary-adrenal (HPA) axis represent two of the main systems implicated in stress adaptations. Morphine withdrawal produces an activation of HPA axis in rats due to an activation of NTS-A2 noradrenergic cell group. There are evidences supporting that CRF neurons in the PVN innervate noradrenergic brain stem nuclei and also the existence of a NA-CRF loop in which CRF would activate brainstem noradrenergic activity, which in turn activate PVN CRF activity, effectively closing the loop. Previous studies from our laboratory have shown a significant attenuation of noradrenergic hyperactivity during morphine withdrawal in adrenalectomized rats. Thereby, the aim of present work was to investigate the effects of the selective GR antagonist mifepristone on morphine withdrawal-induced increased NA turnover in the PVN and on tyrosine hydroxylase (TH) phosphorylation. Male SD rats were made dependent on morphine by sc implantation of two morphine pellets (75 mg). On day 6, rats were pre-treated with vehicle or mifepristone i.p. 30 min before naloxone injection. Sixty minutes later, rats were sacrificed and NA turnover (HPLC) in the PVN was measured. TH phosphorylated at Ser31 (THpSer31) and at Ser40 (THpSer40) expression were quantified by Western Blot in the NTS. Our results showed that the blockade of GR significantly attenuated the increases of NA turnover in the PVN and THpSer31 levels in the NTS-A2 in morphine-withdrawn rats. These data suggest that GR might be involved in the activation of brainstem noradrenergic neurons innervating the PVN during morphine withdrawal.

P43-03

ENVIRONMENTAL CUES ENHANCE CREB PHOSPHORYLATION AND CRF SYNTHESIS IN THE CENTRAL AMYGDALAE

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The brain builds important associations between the environment and its consequences (comfortable or aversive), and this is typically studied using conditioned place preference paradigm (CPP). Conditioning cues, such as walls and floor texture that have previously been paired with reward (e.g. drugs), produce memory changes. These associations can maintain behaviour even without the presence of the drug, and probably involve one of the most important nucleus of the memory: the central amygdale (CeA). CeA is composed by neurons expressing the peptide corticotropin-releasing factor (CRF). CRF was initially identified as a critical component of the stress response with signaling influences in the activity of many diverse brain regions. Recent data indicate that at least some of these behaviours regulated by CRF are mediated through CRF activation of the transcription factor CREB. Swiss male mice were treated with morphine (6 mg/kg) or saline on alternate days. Control group only received saline. One group of animals was conditioned in a room of the CPP apparatus after the corresponding injection and the second one outside the box. An immunohistochemistry of CRF and phosphorylated CREB (pCREB) was made in the CeA. Our results show an increase in the number of neurons expressing CRF and pCREBser133 in the morphine groups in the CeA. Moreover, this increase was more evident in the group conditioned in the CPP apparatus. In conclusion, these results suggest that CRF, pCREBser133 and environment play a role in the memory during drug addiction.

P44-03**EFFECTS OF THE ADMINISTRATION OF CANNABINOID MODULATORS ON COCAINE-INDUCED MOTOR CONDITIONING/BEHAVIOURAL SENSITIZATION AND STEREOTYPED BEHAVIOUR IN MICE**

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Latest studies indicate that endocannabinoid system plays a critical role in the psychomotor functions and addiction. In fact, a new hypothesis has been postulated in the regulation of the rewarding effects of abused drugs. Traditionally, dopamine has been associated with the reinforcing effects of stimulant drugs, behavioral studies in rodents indicate the possibility that endocannabinoids are involved in acute/chronic effects of drug abuse, addictive behaviours and the psychostimulant addictive process. Modulation of cannabinoid receptors might block the direct reinforcing effect or prevent the relapse to dopamine. The purpose of the study was to analyse the effects of pharmacological manipulation with cannabinoids modulators on cocaine-induced motor conditioning/behavioural sensitization and on quinpirole-induced hyperactivity/stereotypy in mice. Cocaine conditioning (20 mg/kg) increased locomotor activity during 5 days and provoked a significant increase in conditioned locomotion, which was inhibited by injection of WIN55, 212-2 (2–4 mg/kg). Cocaine sensitization (10 mg/kg) was blocked by administration of WIN55, 212-2 (4 mg/kg) and HU210 (0.05 mg/kg). On the other hand, administration of the D (2) receptor agonist quinpirole produced hyperlocomotion and stereotyped behaviours. A prior injection of FAAH/MGL inhibitors reduced the stimulation of motor behaviours, blocking the stereotyped behaviours and decreasing quinpirole-elicited hyperlocomotion, but they had no effect either in stereotypes or in locomotion when injected alone. These results support a primary role of the endocannabinoid system in the regulation of dopaminergic transmission-mediated psychomotor activity induced by cocaine. The endocannabinoid system could be explored as a potential drug target for the treatment of pathologies related to psychomotor over excitability.

P45-03**MODULATION OF SEROTONIN NEUROTRANSMISSION BY INDIRECT STIMULATION OF ENDOCANNABINOID SYSTEM: A POSSIBLE ROLE IN DEPRESSION**

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It has been described that FAAH (fatty acid amine hydrolase) inhibitors exhibit antidepressant-like effects. The therapeutic effects of antidepressant drugs might be related to adaptive changes in the elements of serotonergic neurotransmission, including 5-HT_{1A} receptors or serotonin transporter. The aim of this study was to extend our knowledge on the crosstalk mechanisms between endocannabinoid and serotonergic systems and their implication in depression. Male mice were treated with vehicle (1% DMSO i.p.), fluoxetine (160 mg/l p.o.), URB597 (0.3 mg/kg i.p.) and their combination during 28 days and the experiments were carried out 24 h later. The density of serotonin transporter decreased in dorsal raphe nucleus (DRN) as well as in the projection areas of 5-HT neurotransmission after chronic fluoxetine whereas URB597 did not have significant effect. In the DRN, all treatments decrease the efficacy of 8-OH-DPAT-induced stimulation (red = 62.3% for fluoxetine) (red = 35.6% for URB597) of [³⁵S]GTPγS binding without changes in 5-HT_{1A} receptor density. To confirm the desensitization of 5-HT_{1A} autoreceptors we also investigated the 8-OH-DPAT-induced hypothermia. Chronic fluoxetine (red = 65.4%) and its association with URB597 atten-

uated 8-OH-DPAT-induced hypothermia; however URB597 (red = 15.4%) administration only partially reversed this effect. Our data indicate that chronic URB597 induces some neurochemical adaptative changes in serotonin neurotransmission similar to those found with fluoxetine. However, the combination of the FAAH inhibitor with the antidepressant does not result in a potentiation of the response, at least in this experimental approach.

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P46-03**GLUCOCORTICOIDS REGULATION OF ΔFOSB EXPRESSION IN TH AND DYNORPHIN NEURONS DURING MORPHINE DEPENDENCE**

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It is known that several brain regions are dysregulated during drug addiction, including the brain stress system. Noradrenaline and dynorphin (DYN) are important neurotransmitters modulating this neurocircuitry. Glucocorticoids (GC) have been involved in specific aspects of drug addiction process, acting as transcription factors, thus regulating the expression of target genes. Changes in gene expression patterns are important mechanism by which drugs of abuse causes long-lasting alterations in the brain. ΔFosB is a member of the Fos family of transcription factors that gradually accumulates with repeated drug exposure in different brain regions. The aim of the present study was to reveal the role of GC on ΔFosB protein expression both in DYN neurons from the nucleus accumbens shell [NAc(shell)] and the noradrenergic neurons from the nucleus of the solitary tract (NTS). Male Sprague-Dawley rats underwent sham adrenalectomized (SHAM) or bilaterally adrenalectomized (ADX) and were rendered dependent on morphine for 10 days. Using immunohistochemical procedures, the expression of FosB/ΔFosB in tyrosine hydroxylase (TH)-positive neurons and in pro-DYN-positive neurons was measured. The number of ΔFosB+/pro-DYN+ neurons in ADX morphine-dependent rats was significantly lower regarding to sham morphine-dependent animals. We also found a significant increase in the number of ΔFosB+/TH+ neurons in the NTS from sham morphine-treated rats, which was not observed in ADX morphine-dependent rats. Present data suggest that GC would play a main role in the regulation of ΔFosB expression in specific neural populations of the NAc(shell) and NTS.

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P47-03**RGS14(414) GENE DELIVERY INTO BRAIN AREA V2 INDUCES BOTH THE RECOVERY AND PREVENTION OF MEMORY LOSS IN AGEING AND ALZHEIMER'S DISEASE**

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Deficits in memory function are not only comorbid with many psychiatric and neurological disorders but also accompany normal ageing. Intact memory function is critical to carry out daily life activities and therefore, cognitive enhancement pharmacological agents are viewed as a strategy to treat memory deficits. However, available stimulants and drugs have failed to produce therapeutic efficacy in humans. More effective and precise therapeutic strategies are needed. Recently, we observed that the gene delivery of a regulator of G-protein signaling 14 of 414 amino acids (RGS14(414)) into layer 6 neurons of area V2 of secondary visual cortex

enhanced visual memory to such extent that it led to the conversion of short-term memory of 45 min into long lasting long-term memory. Here, we have tested if whether this RGS14(414) protein can prevent and/or reverse a declarative memory loss in two most representative models, normal ageing and Alzheimer's disease, where memory deficit has consistently been observed. We found that expression of RGS14(414) protein into the layer 6 neurons of area V2 not only reversed a declarative memory deficit in ageing rats and transgenic mice model of Alzheimer's disease but also prevented its onset in both models. These findings indicate that RGS14(414) protein-mediated activation of area V2 neurons is adequate to amend the memory loss seen in both ageing and Alzheimer's disease, and the combination of RGS14(414) protein and its targeted expression into area V2 could serve as a potential strategy for the treatment of declarative memory deficits in patients.

P48-03 CONTROLLING HOUSING CONDITIONS IN OBX MICE IS CRUCIAL TO SUCCEED IN THE SCREENING OF ANTIDEPRESSANT AND ANXIOLYTIC DRUGS

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Housing conditions may influence the response of rodents on behavioural paradigms, helping or hindering the identification of anxiolytic and antidepressant effects of drugs. We have evaluated the behavioural consequence of social isolation on an animal model of comorbid depression and anxiety, the bilateral olfactory bulbectomy (OBX) in mice. C57BL/6 mice were OBX- or sham-operated and divided into four groups: OBX-grouped (OBX-G), OBX-isolated (OBX-I), sham-grouped (Sham-G) and sham-isolated (Sham-I). Behaviour was evaluated in the open-field test (OFT), sucrose-intake, novelty-suppressed feeding (NSF), forced-swimming test (FST) and DOI-induced head-twitch response. In the OFT, OBX-induced hyperactivity and increased exploratory behaviour were enhanced by isolation (+41% and +61% respectively vs. OBX-G). Bulbectomy-induced anxiety was less apparent in OBX-isolated mice, due to the increased basal level of anxiety showed by the Sham-I counterparts (+69% vs. Sham-G). In the NSF and FST the hyperactivity exhibited by OBX-mice biased the results when compared with sham animals. However, isolation increased the latency to feeding in the NSF in both OBX (+109%) and Sham mice (+84%). In the sucrose-intake test, OBX-induced anhedonia was not affected by housing conditions. Finally, the number of DOI-induced head-twitches was increased only in OBX-I mice (+38%). For the evaluation of antidepressant effects, isolation in OBX mice provides an improved model of animal depression. For testing anxiolytic properties of drugs, both the isolation and the OBX on their own would be useful models.

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P49-03 IN VITRO CHARACTERIZATION OF AN IMMORTALIZED MÜLLER GLIAL CELL LINE EXPRESSING STEM CELL MARKERS FROM ADULT MOUSE

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Retina in adult mammals, unlike those in lower vertebrates, is not known to support neurogenesis. However, growing evidence suggest that neurogenic potential may be conserved, and therefore could be exploited for a cell-based regenerative therapy to restore visual function [1,2]. Moreover, data show that glial cells may have a role as neural precursors in the adult central nervous system [1-3]. So, generation and characterization of novel Müller glial cell lines from the postnatal retina may represent a valuable tool for development of new therapeutic approaches. An immortalized mouse cell line was obtained from Müller cells of C57BL/6 adult mice retinas following previously described methods [4,5]. The expression of the Müller cell markers Vimentin, CRALBP and GFAP as well as the progenitor and stem cell markers Nestin, Abcg2, α -tubulin, β -III-tubulin, Chx10, Pax6 and Notch1 was characterized by RT-PCR and/or Western-blot analysis. The ability of cells to form neurospheres was also tested [5]. Results showed that (i) the cell line expresses the Müller marker Vimentin and the progenitor and stem cell markers Nestin, Abcg2, α -tubulin and β -III-tubulin, whereas lacks CRALBP, GFAP, Chx10, Pax6 and Notch1 markers, and (ii) the cells were able to develop neurospheres. Altogether these results indicate that a cell line expressing both neural and stem cell markers was isolated, with potential interest in regeneration studies.

References:

1. Das et al. Dev Biol 2006;299:283-302.
2. Lawrence et al. Stem cells 2007;25: 2033-2043.
3. Otteson and Phillips. IOVS 2010;51:5991-6000.
4. Hicks and Courtois. Exp Eye Res 1990;51:119-129.
5. Florian et al. Biochem Biophys Res Comm 2008;374:187-191.

P50-03 VASCULAR DYSFUNCTION IN A TRANSGENIC MODEL OF ALZHEIMER'S DISEASE: EFFECTS OF CANNABINOID AGONISTS

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Alzheimer's disease (AD) is characterized by increased deposition of β -amyloid (A β), neurofibrillary tangles, loss of subsets of neurons and glial activation. A β accumulation occurs in senile plaques and cerebrovascular deposits. There is evidence of altered vascular function in AD and its transgenic models. Cannabinoids, neuroprotective and anti-inflammatory agents, induce vasodilation *in vivo* and *in vitro*. We have demonstrated a beneficial effect of cannabinoids in models of AD by preventing glial activation. Now we study the effects of these compounds in amyloid precursor protein (APP) transgenic mice, line 2576, and on A β altered vascular responses in isolated ring aortae.

Results: We found an increased density of collagen IV positive vessels in AD frontal cortex and in 12 months old Tg APP mice. In APP Tg mice aortae, phenylephrine and thromboxane agonist U46619 vasoconstriction was significantly increased, and no change in vasodilatation to acetylcholine (ACh) was observed. WIN 55,212-2 (CB1/CB2 agonist), and JWH-133 (CB2 selective agonist), caused a dose-dependent vasodilatation in wild type mice, significantly reduced in Tg APP. A β incubation reduced ACh-induced relaxation; cannabinoids counteracted this effect. At the ultrastructural level Tg APP aortae were similar (e.g. endothelial cells, mitochondria or muscle cells), although, had increased collagen.

Conclusions: We confirm and extend the existence of altered vascular responses in Tg APP and in A β treated vessels. Tg APP displayed decreased vasodilatation to pharmacologically different cannabinoid agonists, which prevent decreased ACh vasodilatation in presence of A β . Suggesting that treatment with cannabinoids may ameliorate the vascular responses in AD-type pathology.

P51-03**ACTIVATION OF ENDOCYTOSIS AND L CALCIUM CHANNELS BY β -AMYLOID PEPTIDE: ROLE OF SPHINGOLIPIDS**

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Alteration of calcium homeostasis and the elevation of the intracellular Ca^{2+} [Ca^{2+}]_i level have been suggested to be responsible for the toxic effects of amyloid- β peptide (A β). The exact mechanism by which A β increases [Ca^{2+}]_i is controversy. The present study explored the effect of A β 25-35 on calcium influx, exo-endocytosis on chromaffin cells. Calcium currents (ICa) and membrane capacitance (Cm) were measured under the perforated patch configuration of the patch-clamp technique. Cells were constantly perfused with a standard Tyrode solution and internally dialyzed with a perforated-patch solution containing (in mM): 135 CsGlutamate, 10 HEPES, 9 NaCl, pH 7.2 adjusted with CsOH. Electrophysiological data were carried out using an EPC-9 amplifier under the control of Pulse software (HEKA Elektronik). Cell membrane capacitance (Cm) changes were estimated by the Lindau-Neher technique applying a sinusoidal wave function (1 kHz, 80 mV peak to peak amplitude) before and after the depolarizing pulse. Cells were held at -80 mV, and single 200-ms depolarising pulses to voltages where ICa peak was reached were applied at 2-min intervals. A β treatment increased ICa by two-fold, an increase that was blocked by nifedipine. A β treatment also increased the endocytic response which was blocked both by L and non-L calcium channels blockers. The inhibition of SMase by amitryptilin and that of caveolin by genistein (experimental approaches used to inhibit endocytic pathways) reduced the endocytosis and prevented the increase of the calcium influx through VDCCs after A β treatment. Our data suggest that endocytic mechanism and VDCCs run parallels in the toxicity of the A β peptide.

P52-03**RGS14(414) PROTEIN ENHANCES ENCODING OF BOTH OBJECT AND SPATIAL MEMORY IN PERIRHINAL CORTEX**

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Within the medial temporal lobe (MTL), perirhinal cortex (PRh) is thought to be essential for processing of object visual memory (OVM). PRh receives the object information input through ventral stream areas, whereas spatial information input to the postrhinal cortex comes from dorsal stream areas. It is believed that information processing in ventral and dorsal streams remains largely segregated and that they do not converge within the PRh. In imaging experiments, PRh was activated significantly more by pictures of objects and pictures of novel spatial arrangements produced no effect. Thus, PRh is important in OVM and not in spatial memory. Recently, we observed that the overexpression of a regulator of G-protein signaling 14 of 414 amino acids (RGS14(414)) into area V2 of visual cortex led to the conversion of short-term OVM of 45 min into long lasting long-term OVM. However, it remains to be tested whether this protein can produce memory enhancement effect into PRh. Therefore, we have explored the effect of overexpression of RGS14(414) protein into PRh on both object and spatial memory. Our results show that RGS14(414) protein into PRh not only promoted enhancement in object memory but also boosted the spatial memory in rats. In contrast, expression of RGS14(414) in area V2 showed no effect on spatial memory. Thus, in addition to the role of PRh in the formation of both object and spatial memory, these findings suggest that RGS14(414) protein might activate a pathway common for both object and spatial components of visual memory.

P53-03**NEW TETRAHYDROISOQUINOLINES AS DOPAMINERGIC LIGANDS: SYNTHESIS, MOLECULAR MODELING STUDY AND ANTIDEPRESSANT-LIKE BIOLOGICAL ACTIVITY**

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New tetrahydroisoquinolines as dopaminergic ligands: synthesis, antidepressant-like biological activity and molecular modeling study. Dopamine-mediated neurotransmission plays an important role in several psychiatric and neurological disorders affecting several million people worldwide. Substituted isoquinolines represent a class of natural and synthetic compounds that has been evaluated for their ability to inhibit the dopamine transporter. Two series of halogenated 1-benzyl-7-chloro-6-hydroxy-tetrahydroisoquinolines and one serie of 1-butyl-tetrahydroisoquinoleine were prepared to explore the influence of each serie on the affinity for dopamine receptors. All the compounds displayed a high affinity for D1-like and/or D2-like dopamine receptors in striatal membranes. The halogen placed on the benzylic ring in 1-benzyl-THIQs, compounds of the series 1, 2'-bromobenzyl derivatives with Ki values into the nanomolar range, and the series 2, 2', 4'-dichlorobenzyl-THIQ homologues, proves to be an important factor to modulate selectivity at dopamine receptor. In addition, one of the 1-butyl-tetrahydroisoquinoleine compounds was evaluated *in vivo* using behavioural assays in mice. This compound increased locomotor activity in a large dose range. Furthermore, this lead compound produced reduction in immobility time in forced swimming test at a dose that did not modify locomotor activity. A molecular modeling study on the BTHIQs acting as dopaminergic ligands was carried out. A significant correlation between binding energies obtained from DFT calculations and experimental pKi values was obtained predicting the potential dopaminergic effect of non-synthesized BTHIQs.

P54-03**A LACK OF CORRELATION BETWEEN SGI2 PROTEIN AND NEURONAL APOPTOSIS DURING RAT BRAIN DEVELOPMENT**

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Treatment with dopamine and other dopamine D2 receptor agonists has been shown to induce cell death through activation of caspase-3 pathway. However, initial step that leads to the activation of caspase-3 in D2 receptor-mediated apoptotic pathway remains unclear. Recently, we have shown that a spliced variant of G α i2 protein (sG α i2) forms intracellular complex with D2 receptors by protein-protein interaction and that D2 drugs treatment causes the liberation of sG α i2 protein from complex. This unbound form of sG α i2 protein was able to activate caspase-3 pathway in baby hamster kidney (BHK) cells. Expression of sG α i2 protein in these cells led to the production of active form of caspase-3 and activation of p38 mitogen-activated protein kinase (p38 MAPK) and extracellular regulated kinase 1/2 (ERK1/2). Co-expression of sG α i2 with either D2 short (D2S) or D2 long (D2L) isoforms of dopamine D2 receptors-blocked the activation of caspase-3 pathway. These results demonstrated that high level of unbound sG α i2 protein can promote the cell death and engagement of this protein with D2 receptors can prevent this activity. Thus, it was proposed that balance between sG α i2 protein and D2 receptor plays a critical role in cell death. We have tested this theory in rat during brain development period, a model known for high rate of apoptosis. We found that the proportion of D2 receptor abundance was higher than sG α i2 protein even when the active caspase-3 level was higher. These observations suggest no relationship of caspase-3 activation with either sG α i2 protein or D2 receptor during rat brain development.

P55-03**THIOPHENE AND FURANE DERIVATIVES AS NEW PDE7 INHIBITORS OR PUTATIVE DRUGS FOR CNS DISORDERS**

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Phosphodiesterases (PDEs) play a critical role in various biological processes by hydrolyzing the adenosine and guanosine 3',5'-cyclic monophosphate nucleotides (cAMP and cGMP, respectively) to the corresponding 5'-monophosphate nucleotides. Inhibition of PDE activity produces an increase of cAMP and cGMP intracellular levels that activates specific protein phosphorylation pathways involved in a variety of functional responses (Palacios et al. *Farmacologia* 1995;50:819–827). To date, eleven families have been identified and they mediate the action of many hormones, neurotransmitters, and other cellular effectors (Lugnier et al. *Pharmacol Ther* 2006;109:366–398). Several reports suggest that PDEs are new targets for CNS diseases. Virtually all PDEs are expressed in the CNS, making this family particularly attractive as source of new targets for the treatment of psychiatric and neurodegenerative disorders. Moreover, PDEs inhibitors emerge as promising new drugs for the treatment of dementia, cognitive disorders, depression and/or schizophrenia (Menniti et al. *Nat Rev Drug Discov* 2006;5:660–670.) In the present study we have evaluated the inhibitory effect of a family of heterocyclic compounds (thiophene and furane derivatives) in PDE7. To determine this inhibitory activity we have used a scintillation proximity assay for the direct detection of [³H]-labelled AMP. The activity of the compounds varied from inactive to compounds with low μM potency. From the compounds assayed we identified compounds MR1.51 and MR2.36 with IC₅₀ of 5.1 and 3.2 μM respectively. These compounds are considered the best hits for further optimization.

P56-03**LYMPHOBLASTOID CELL VIABILITY, A NOVEL TOOL FOR FARMACOLOGICAL EFFECT PREDICTION**

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Schizophrenia is a chronic mental illness that affects approximately 1% of the population. Current drugs for the treatment of schizophrenia are divided in the typical antipsychotics (first generation) such as haloperidol and atypical (second generation) such as clozapine. The latter was discovered over 50 years ago and remains the 'gold standard' atypical antipsychotic drug inducing fewer extrapyramidal effects [1] and showing efficacy in the treatment resistant schizophrenia [2]. Clozapine has a complex pharmacological profile [3] which makes it difficult to predict antipsychotic activity before performing *in vivo* studies. In order to predict the clinical efficacy it has recently been reported that drugs can be classified according to the modulation of cell viability [4]. We hypothesized that differences in cell viability after drug treatment could allow to predict the antipsychotic profile of drugs. We have used human lymphoblastoid cells and assessed the inhibition of their growth after a 72 h treatment with a single dose of either antipsychotics typical (haloperidol, 33 μM) or atypical (clozapine, 33 μM). We observed a good correlation between the cell viability of the different cell lines often both treatments ($R^2 = 0.925$, $R^2 = 0.856$) which indicates that we are able to predict an antipsychotic efficacy by this methodology. Furthermore we observed a higher growth inhibition with haloperidol treated cell lines also those treated with clozapine. These differences would also allow to discriminate between typical and atypical antipsychotics.

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References:

1. Farah A. *Prim Care Companion. J Clin Psychiatry* 2005;7:268–274.
2. Kane J. *Arch Gen Psychiatry* 1988;45:789–96.
3. Roth BL et al. *Nat Rev Drug Discovery* 2004;3 (4):353–359.
4. Gurwitz D et al. *Pharmacogenomics* 2010;11 (3):327–340.

P57-03**INFLUENCE OF REELIN EXPRESSION ON THE CONFORMATIONAL DISTRIBUTION OF 5-HT_{2A} RECEPTORS**

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Reelin is an extracellular matrix glycoprotein related to neuronal plasticity, stability of dendritic spines (PNAS 2001;98:3477–824), and membrane proteins clustering (Mol Cell Biol 2004;24:1378–865). A decrease in reelin expression (40–50%) was observed in schizophrenic patients (PNAS 1998;95:15718–232, Mol Psych 2000;5:633–654) and in the heterozygous reeler mouse (HZ). In previous studies we have shown the influence of the levels of reelin in 5-HT_{2A} receptors expression (Varela MJ. Doctoral Thesis, USC 2011). We hypothesized that the levels of reelin could influence the conformational distribution of 5-HT_{2A} receptors. We carried out competition assays with haloperidol, clozapine and risperidone against [³H] LSD in mice synaptosomal membranes from reeler (HZ) and wild type mice (WT). Haloperidol and risperidone displayed biphasic competition curves in HZ while clozapine has a monophasic competition profile in both genotypes. No significant differences in the K_i values of haloperidol and clozapine were observed but a decrease of risperidone K_i values was observed at HZ reeler mice (K_i high: 1.56 ± 0.23 nM, K_i low: 555.45 ± 235.65 nM) with respect to WT (K_i high: 0.37 ± 0.16, K_i low: 1315.25 ± 73.75 nM). Evaluating structurally related pharmacological tools we observed that QF0703B and QF1004B have monophasic competition profiles, while QF0108B has biphasic competition curves with K_i values (K_i high: 0.72 nM, K_i low: 327 nM) close to that observed with risperidone. In summary, our results suggest a reelin and ligand-dependent conformational distribution of 5-HT_{2A} receptors. Grants: MICINN (SAF2009-13609-CO4-01)

P58-03**ANTIOXIDANT, ANTI-INFLAMMATORY AND NEUROPROTECTIVE PROFILE OF COUMARIN-RESVERATROL HYBRID DERIVATIVES**

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Neuroinflammation has been known to play a critical role in the pathogenesis of chronic neurodegenerative diseases. Taking into account the anti-inflammatory effects of the trans-resveratrol, the biological properties of some synthetic coumarin-resveratrol hybrid derivatives were studied. 3,4-double bond of the coumarin nucleus fixes the trans disposition of the trans-resveratrol and some modifications of the position of its hydroxyl groups were performed. COX activity was evaluated using a chromogenic assay based on the oxidation of TMPD during the reduction of PGG₂ to PGH₂. Enzyme assay peroxidase activity was measured by spectrophotometry, with the chromogen ortho-phenylenediamine. Nitrite production was assayed by the Griess reaction. The production of ROS was investigated using OxyBURSTR Green probes. A DPPH radical scavenging assay was carried out according to a published procedure (Kim et al. 2002). Fluo-4 was used for Ca²⁺ measurements. LPS has been employed to induce microglial activation in culture cells from 20 days old rat embryos cerebral cortex. Some derivatives showed a

potent COX inhibition as well as the peroxidase activity. They also resulted in free radical scavengers and inhibitors of ROS production. Most of them modulated intracellular Ca^{+2} concentrations. Finally, they also were able to revert damage induced by microglia activation in neuronal culture. We have developed coumarin-resveratrol hybrid derivatives that display interesting *in vitro* biological activities for the treatment of neurodegenerative diseases: antioxidant, anti-inflammatory and neuroprotective properties.

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P59-03

SYNTHESIS OF HYDROXYTYROSOL NITRODERIVATIVES AS COMT INHIBITORS

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It is well known that hydroxytyrosol (HTy), a polyphenolic compound of virgin olive oil, shows a marked role as antioxidant in oxidative processes. Moreover, HTy has been shown to prevent oxidative injuries in animal models as well as HepG2 and Caco-2 cell lines. In the present study, we propose the synthesis of new antioxidant compounds derived from HTy. The aim of this study is to obtain nitroderivatives of HTy and to evaluate their antioxidant activities. In addition, it is known that several nitrocatechol derivatives act as COMT inhibitors and, some of them are being now used in the Parkinson therapy. In this sense, NO₂HTy, NO₂HTyAc and EtNO₂HTy were synthesized and purified by column chromatography. Optimum conditions were used for the synthetic process with acceptable overall yields. Further, COMT inhibition assay was carried out using five groups: healthy control (C), commercial RO-41 inhibitor (I), nitrohydroxytyrosol (NO₂HTy), nitrohydroxytyrosyl acetate (NO₂HTyAc) and ethyl nitrohydroxytyrosol (EtNO₂HTy). Dopamine, DOPAC, and HVA levels in brain were measured by high performance liquid chromatography (HPLC). The findings demonstrated that Dopamine and DOPAC levels were significantly increased in nitrohydroxytyrosol group compared to control group and even, to commercial RO-41 inhibitor in the case of Dopamina. We could not achieve conclusive results for HVA.

Conclusion: The synthesis of nitroderivatives of HTy provides an approach as COMT inhibitors.

P60-03

ANTIOXIDANT AND NEUROPROTECTIVE EFFECTS OF 3,4 DIHYDROXYPHENYLGLYCOL IN RAT BRAIN SLICES

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Hydroxytyrosol, the most abundant polyphenol in virgin olive oil, has a clear antioxidant and neuroprotective effect, focusing on the first effect the main mechanism by which it exerts its effect on nerve tissue. There

are some doubts about whether other phenolic compounds present in olives and virgin olive oil possess some biological activity, especially in terms of its antioxidant capacity. There is evidence that the 3, 4 dihydroxyphenylglycol (DHPG) has shown an antioxidant effect on chemical matrices even higher than the hydroxytyrosol. The aim of this study was to evaluate the antioxidant and neuroprotective potential of DHPG in rat brain slices subjected to hypoxia-reoxygenation. The study was performed *in vitro* by incubating different concentrations of the compounds from the beginning of the experiment. Lipid peroxidation (TBARS) induced by ferrous salts in brain membranes of male Wistar rats was measured. In the hypoxia-reoxygenation model TBARS and LDH efflux were quantified. DHPG inhibits ferrous-induced lipid peroxidation (IC₅₀ μM : 68.9 \pm 6.7 vs. 77.8 \pm 5.6 for hydroxytyrosol). In the hypoxia reoxygenation model DHPG inhibited LDH efflux 32% with respect to control and hydroxytyrosol 72.3%. In this model TBARS was inhibited 6.7% with DHPG and a 59.7% with hydroxytyrosol. Although DHPG has antioxidant and neuroprotective effect, this effect is lower than the one with hydroxytyrosol.

P61-03

IMPORTANCE OF TYPE-2 CYCLOOXYGENASE ACTIVITY INHIBITION IN NEUROPROTECTION

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A neuroprotective effect of the non-steroidal anti-inflammatory drugs (NSAID) has been demonstrated, especially in neurodegenerative diseases. The cyclooxygenase (COX) activity has been related with neuronal damage. However the role of the two COX isoenzymes has not been elucidated. The aim of the study is to evaluate the importance of type-2 cyclooxygenase (COX-2) activity inhibition in neuroprotection. We used celecoxib as a selective COX-2 inhibitor, acetylsalicylic acid (ASA) as COX-1 inhibitor and ibuprofen as non selective COX1/2 inhibitor. We used an *in vitro* model of hypoxia-reoxygenation in rat hippocampus slices (n = 6 rats per group). After reoxygenation period we measured: LDH as indirect index of neuronal death, brain PGE₂ and brain interleukin 1 β and 10 as inflammatory mediators. Oxidative and nitrosative stress were defined by TBARS, GSH, GSSG, 3-nitrotyrosine and nitrite/nitrate production. The LDH efflux (IC₅₀ μM) values obtained after reoxygenation of brain slices were for ASA 929.2 \pm 61.2, celecoxib: 8.93 \pm 1.43 and ibuprofen 33.9 \pm 13.1. The inflammatory mediators expressed as percentage respect to control obtained with neuroprotective IC₅₀ concentrations were as follows, PGE₂: -79.1%, -10.4%, and -51.45% for ASA, celecoxib and ibuprofen respectively; IL 1 β : +31%, -5.02% and -41.22%; IL 10: 0%, +18.63% and +10.25%. For the nitrosative stress: +27.1% for ASA, -52.5% for celecoxib and -36.54 for ibuprofen. The COX-2 inhibition seen not been the main mechanism for the neuroprotective effect of NSAID in this experimental model.

NATURAL PRODUCTS

P62-04

UNSAAPONIFIABLE FRACTION OF EXTRA VIRGIN OLIVE OIL INHIBITS CELL GROWTH IN HT-29 HUMAN COLON ADENOCARCINOMA CELLS: MECHANISMS AND SIGNALING PATHWAYS

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Background: Extravirgin olive oil (EVOO) is the essential component of the Mediterranean diet. EVOO has demonstrated a great functional versatility related to its antiproliferative and anti-inflammatory properties. Although traditionally, many beneficial properties has been ascribed to its high oleic acid content. Nowadays, it is clear that many of the EVOO beneficial effects may be due to its minor components present in the Un-saponifiable Fraction (UF). We studied UF effects in HT-29 human colon adenocarcinoma cells and characterized whether UF-mediated cell growth inhibition was accompanied by induction of apoptosis. Involved molecular mechanisms in its potential proapoptotic effects were also studied.

Methods: FI was isolated by means of liquid-liquid partition. Quantitative analysis was carried out by HPLC. Cell growth and viability assays were determined by SRB test at different time points (24, 48 and 72 h). The proapoptotic effect was evaluated by flow cytometric studies. The expression of COX-2, p53 and mitogen-activated protein kinases (MAP-Ks) p38, JNK, p-ERK1/2 were determined by immunoblotting.

Results: We found that microgram per millilitre concentrations of UF, significantly reduced the growth of HT-29 cell line. Lower doses of 250 µg/ml of fraction exhibited no significant effect on cell growth. UF-mediated cell growth inhibition was accompanied by induction of apoptosis showing significant increase in the number of early and late apoptotic cells in presence of 250 µg/ml of UF. Besides, an up-regulation of p53 protein and down-regulation of COX-2 in UF-treated cells were observed. In relation of MAPKs pathway, we detected no changes in protein expression.

Conclusion: These novel findings suggest that UF from EVOO may exert chemopreventive effects in colorectal cancer through cell death-mediated mechanisms.

P63-04

DETERMINATION OF THE ANTIOXIDANT ACTIVITY OF *SIDERITIS HYSSOPIFOLIA*

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Introduction: A large number of medicinal plants and their purified extract have shown beneficial therapeutic potentials. The aim of this study was to evaluate the antioxidant activity of the ether, methanol, chloroform and butanolic extracts obtained from the aerial parts of *Sideritis hyssopifolia* using DPPH method and ascorbic acid as the standard antioxidant.

Material and Methods: For each extract and ascorbic acid different concentrations were tested. Extracts (ether, methanol, chloroform and butanolic) and ascorbic acid were dissolved in methanol. 0.1 ml of this solution was added to 3.9 ml of methanol DPPH solution (0.1 mM). The absorbance was determined at 517 nm at 0, 15, 30, 60, 90 and 120 min in the dark at room temperature. The inhibitory concentration by 50% (IC₅₀), defined as the antioxidant concentrations that caused 50% loss of DPPH activity, was calculated from the regression line obtained by representing the inhibition percentage against the concentration of the corresponding extract or standard.

Results and Conclusion: The reaction time for DPPH was on 30 min. The results have shown that the IC₅₀ values obtained for the different extracts and standard antioxidant tested were: ascorbic acid.- 3.574 µg/

ml; butanolic extract.- 13.838 µg/ml; methanol extract.- 35.560 µg/ml; ether extract.- 48.019 µg/ml and chloroform extract.- 401.858 µg/ml. From the results obtained in the present study, we can conclude that the best antioxidant activity was found in the butanolic extract, relative to our standard antioxidant, ascorbic acid.

P64-04

EVALUATION OF THE ANTIOXIDANT ACTIVITY OF THE BUTANOLIC EXTRACT OBTAINED FROM *SIDERITIS HYSSOPIFOLIA*

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Introduction: The butanolic extract obtained of the aerial parts of *Sideritis hyssopifolia* is rich in polyphenolic compounds. The aim of this study was to evaluate the antioxidant activity of the butanolic extract using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) methods and ascorbic acid as an antioxidant standard.

Material and Methods: DPPH method: after addition of different butanolic extract and ascorbic acid concentrations to DPPH (0.1 mM), the percentage of remaining DPPH was determined at different times measuring the absorbance at 517 nm. ABTS method: after addition of different butanolic extract and ascorbic acid concentrations to ABTS●+ (ABTS 7 mM with potassium persulphate 2.45 mM), the antioxidant activity was determined at different times measuring the absorbance at 734 nm. Extract or standard concentration providing 50% inhibition (IC₅₀) was obtained by plotting the percentage inhibition against extract or standard concentration.

Results and Conclusion: The results indicate that the reaction time for DPPH was on 30 min and for ABTS was on 7 min. The IC₅₀ values calculated using DPPH method were 13.838 µg/ml for butanolic extract and 3.574 µg/ml for ascorbic acid, and calculated using ABTS method were 4.755 µg/ml for butanolic extract and 1.959 µg/ml for ascorbic acid. It was observed a good correlation between DPPH and ABTS radical scavenging assays. Both methods were valid to calculate antioxidant activity of butanolic extract although ABTS method takes less time and required less concentration of butanolic extract and ascorbic acid.

P65-04

BIOACTIVE COMPOUNDS ISOLATED FROM *CYSTOSEIRA USNEOIDES* WITH ANTICANCER, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES

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Marine algae are an inexhaustible source of chemical compounds that produce a wide variety of biologically active secondary metabolites. Macroalgae have become an important target for the biotechnology industry because of the large number of bioactive compounds recently discovered from them. The objective of this study was to examine the anticancer, antioxidant and anti-inflammatory activities of extracts and isolated compounds from the seaweed *Cystoseira usneoides*, collected from the northern mediterranean coast of Morocco. Chemist studies have

been done in collaboration with Department of Chemistry, Faculty of Marine Sciences and Environmental, University of Cadiz, following a bioguided procedure. Antioxidant activities were assessed following the 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) method. Cytotoxic studies were determined by sulforhodamine (SRB) method, and apoptosis and cell cycle assays by flow cytometry, using HT-29 colon cancer cells. Anti-inflammatory activity was calculated measuring the production of the TNF- α cytokine by ELISA, using the THP-1 cell line. We identified over six pure compounds nominated meroterpenoids, and the results of the biological studies showed that extracts and also meroterpenoids compounds isolated from *Cystoseira usneoides* displayed a high antioxidant activity. Similarly, *Cystoseira usneoides* and isolated compounds exhibited potent cytotoxic activity against HT-29 cell line. The current study demonstrates the anticancer properties of meroterpenoids compounds accompanied by a very significant antioxidant effects. Further studies are required deepening in antitumor properties of this interesting algae from Mediterranean coast.

P66-04

ANTINOCICEPTIVE AND ANTIDIARRHOEAL ACTIVITY OF *SPHAERALCEA ANGUSTIFOLIA*

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Introduction: *Sphaeralcea angustifolia* (Malvaceae), is used in Mexican Traditional Medicine to treat diarrhea, stomach ache and inflammation. We evaluate the ethanol extract for its antinociceptive and antidiarrhoeal activity.

Materials and Methods: The air-dried leaves were ground and extracted by maceration at room temperature with ethanol. After filtration the solvent was evaporated in vacuum to yield crude extract. To evaluate the antinociceptive activity, Male CD1 mice (n = 7) were treated orally at 75, 150, 300, 600 mg/kg doses dissolved in 1 ml of a Tween 80 1% solution in water, or vehicle (Tween 80 1% solution in water) 30 min before intraperitoneal administration of 0.3 ml of 0.5% acetic acid, number of abdominal constriction were recorded in periods of 5 min during 25 min, indomethacin (10 mg/kg) was used as antinociceptive drug. To evaluate the antidiarrhoeal activity, mice were administrate at the same dosis, 30 min before the administration of 1 ml of castor oil, wet stools were recorded during 3 h, loperamide (2.5 mg/kg) was used as antidiarrhoeal drug. All the experimental procedures followed the guidelines on Ethical Standards for Investigations of Experimental pain in animals.

Results: Ethanol extract produced antinociceptive effect dose dependent better than indometacine (P < 0.005), and showed good antidiarrhoeal activity (80.6–100% of inhibition of wet stools).

Conclusion: The results showed analgesic and antidiarrhoeal activities of ethanol extract from *Sphaeralcea angustifolia*. The present studies lend some support to the anecdotal report for the traditional use of *S. angustifolia* in the control of diarrhea and stomach ache.

P67-04

HEPATOPROTECTIVE EFFECT OF *GERANIUM SCHIDEANUM* FOLLOWING THIOACETAMIDE-INDUCED NECROSIS IN RATS

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Introduction: *Geranium shideanum* (Gs) is used by traditional medicine in Central Mexico as an antipyretic and anti-inflammatory. However, scientific evidence does not exist in any literature to corroborate the claim of therapeutic success of Gs in these targets or any other. In the present report the effect of Gs extract were studied in reference to postnecrotic liver damage induced in rats by thioacetamide (TA).

Material and Methods: Rats, pretreated with a single dose of geranium schideanum extract (100 mg/kg) every 24 h for 3 days, were intraperitoneally injected with TA (6.6 mmol/kg) the third day of treatment. Samples of blood and liver were obtained at 0, 24, 48, 72 and 96 h following TA intoxication and parameters related to liver damage like AST, ALT and bilirubin levels were carried out in blood using well established protocols and methods.

Results: The results showed that the pre-treatment with crude extract significantly reduced liver damage. Gs decreased and delayed liver injury by 66% and 70% for AST and ALT respectively at 24 h, the peak of maximum regeneration. Also the levels of bilirubin total were significantly lower in the groups of rats pretreated with Gs. The LD(50) of the plant species obtained was >5000 mg/kg (p.o.).

Conclusion: The data obtained indicate that the crude extract of *Geranium shideanum* has hepatoprotective activity. However, further investigation on the acute toxicity and on the mechanism of the hepatoprotective effect of the plant species needs to be carried out.

P68-04

NEUROPROTECTIVE EFFECT OF ALKYL HYDROXYTYROSYL ETHERS IN RAT BRAIN SLICES SUBJECTED TO HYPOXIA-REOXYGENATION

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As a response to the increasing demand by the food industry for new synthetic lipophilic antioxidants, hydroxytyrosyl (HT) ethyl, butyl, hexyl, octyl and dodecyl ethers have been synthesized from of the naturally occurring antioxidant hydroxytyrosol, with similar or even higher antioxidant activity than free hydroxytyrosol (HT). The aim of the present study was to investigate the possible neuroprotective effect of new type of derivatives in a model of hypoxia-reoxygenation in rat brain slices after *in vitro* incubation. Lactate dehydrogenase (LDH) efflux to the incubation medium was measured as a marker of brain cell death; brain PGE₂ as the product of COX activity; brain IL-1 β and IL-10 as pro- and anti-inflammatory mediators, oxidative and nitrosative stress were defined by TBARS, GSH, GSSG, 3-nitrotyrosine and nitrite/nitrate production. These derivatives inhibited LDH efflux in a concentration-dependent manner, with 50% inhibitory concentrations much lower than starting hydroxytyrosol (HT: 165 μ M; ethyl-HT: 28.17 μ M; butyl-HT: 5.5 μ M; hexyl-HT: 58 μ M; octil-HT: 38.3 μ M and dodecil-HT: 29.1 μ M). TBARS productions was inhibited in a concentration-dependent manner (HT: 77.8 μ M; ethyl-HT: 36.9 μ M; butyl-HT: 5.9 μ M; hexyl-HT: 33.2 μ M; octil-HT: 33 μ M and dodecil-HT: 50.8 μ M). Other inflammatory, oxidative and nitrosative variables were not significantly modified (maximum 20%). Conclusion: The alkyl hydroxytyrosyl ether compounds exert an '*in vitro*' neuroprotective effect in relation with its antioxidant capacity.

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P69-04

GAMMA-ORYZANOL DECREASES NITRIC OXIDE AND TNF- α PRODUCTION BY STIMULATED MURINE MACROPHAGES

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Background: Gamma-oryzanol (OR) a phytosteryl ferulate mixture constituent of rice bran, has a wide spectrum of biological activities as antihyperlipemic, antitumoral or antioxidant [1].

Methods: The study has been focused on the effect of OR on nitric oxide (NO) and TNF- α cytokine release by LPS-stimulated murine macrophage. Nitrite concentration in μM (as index of NO generation) was determined by the Griess reaction. Further the direct nitrite-radical-scavenging effect by OR was evaluated in an *in vitro* experiment using sodium nitroprusside (SNP) as NO donor. TNF- α was quantified by sandwich immunoassay (ELISA).

Results: OR significantly and dose dependently decreased the NO production in LPS-stimulated murine macrophages. The calculated IC_{50} for OR was 24.5 μM . It was found that OR did not directly scavenge nitrite produced by SNP. The TNF- α cytokine production was decreased in an approximately 43% by the highest doses of 100 μM .

Conclusions: These results showed that gamma-oryzanol present in the rice bran in approximately 0.3% [2] has a protective effect against some inflammatory mediators released in this experimental cell model and suggest its potential value as a possible functional or nutraceutical component of the rice bran.

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References:

1. Jin Son M et al. J Clin Biochem Nutr 2010;46 (2):150–6.
2. Ismail M et al. Nutr Metab 2010;7:23.

P70-04

DIURETIC ACTIVITY FROM *ROYSTONEA REGIA* O.F. COOK IN RATS

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Introduction: *Roystonea Regia* O.F. Cook is a tree which belongs to the palmaceas family. It has been traditionally reported that the decoctions from its roots have diuretic properties. The objectives of this paper are to evaluate *in vivo* diuretic activity of a *R. Regia*'s leaves decoction.

Materials and Methods: A decoction with dry roots to the 30% was elaborated and it was given to make rats S/D to a dose of 200, 400 and 800 mg total solids per kg. The volume was completed with fisiologic saline solution to reach an hydrosaline overload with a constant total volume of administration of 40 ml/kg, either for treated groups or for positive controls (furosemide, 20 mg/kg and hydroclorotiazide 10 mg/kg) and negative control (NaCl 0.9%). Urinary excretion volumes were measured in the 1/2, 1, 2, 3, 4, 5, 6 and 8 h and electrolyte concentration Na⁺, K⁺ and normal pH in the total urine collected were determined.

Result: A diuretic action was observed with a dose dependent effect in *R. Regia*'s roots decoction with better results to a dose of 800 mg/kg. All dose levels increased the urinary excretion in a similar way that furosemide does. Sodium and Potassium levels eliminated in urine were significantly higher than the ones in the negative control group.

Conclusion: Diuretic activity was higher with the last dose level (800 mg/kg). pH values of the groups treated with *R. Regia* O.F. Cook increase as the dose increases too, making statistic differences in relation with the negative control and the positive control groups.

P71-04

DIURETIC ACTIVITY OF MINERAL WATERS OF 'FUENTE-REOR' (GRAN CANARIA, CANARY ISLANDS) VS. OTHER WATERS WITH WEAK MINERALIZATION

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Introduction: The spring where Fuentor Water comes from is in Teror (Gran Canary Island). This water was declared as Natural-Mineral-Water in 1995 and classified as water of weak-mineralization. Diuretic activity and physicochemical properties were studied.

Material and Method: Fuentor Water was compared with two mineral waters of weak-mineralization (WWM1 and WWM2) and saline as control. Urinary Volume Excretion (UVE), Maxim Diuresis Time (MDT) and Urinary Concentration (UC) of Na⁺, K⁺ and Cl⁻ in rats were examined.

Results: The ionic concentration in mg/l of Fuentor waters: CO₃H⁻ = 96.80; Cl⁻ = 26.90; NO₃⁻ = 16.20; SO₄²⁻ = 12.20; Ca²⁺ = 14.90; Mg²⁺ = 9.30; Na⁺ = 27.20; K⁺ = 4.80. Dry residue = 215 mg; pH = 6.98. The Fuentor Water produced a steady increase in UVE of water for 6 h following administration (n = 10) (6 h) (UE %: 51.7; 80.1; 91.0; 103.2; 103.5; 108.0 compared with saline (UE %: 11.2; 20.1; 27.9; 33.6; 37.8; 40.4) (P < 0.05) and vs. WWM1 (UE %: 30.2; 62.0; 73.8; 85.2; 86.3; 90.5) (P < 0.05). Fuentor Water produced a significant decrease in MDT (130.4 min) compared with control (188.1 min) (P < 0.05) and vs. WWM1 (160.3) (P < 0.05). On UC of Na⁺, K⁺ and Cl⁻ a significant decrease was found with Fuentor Water of (Na⁺ = 0.17; K⁺ = 0.26; Cl⁻ = 0.23 meq/6 h) vs. saline (Na⁺ = 0.78; K⁺ = 0.43; Cl⁻ = 1.0 meq/6 h). (P < 0.05).

Conclusions: Fuentor Water is bicarbonated-sodic Natural Mineral Water with higher diuretic effects than the other two mineral waters of similar mineralization.

PHARMACOKINETICS

P72-05

INTERSPECIES SCALING FOR ENROFLOXACIN AND MARBOFLOXACIN PHARMACOKINETIC PARAMETERS: VOLUME OF DISTRIBUTION AND CLEARANCE

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Pharmacokinetic parameter prediction by allometric scaling allows both inter and extrapolations between species and dosing calculation. In this study we have used pharmacokinetic parameters from bibliography in order to obtain allometric equations for Volume of distribution (Vdss) and Clearance (Cl) vs. body weight of two fluoroquinolones: enrofloxacin and marbofloxacin on the basis of anatomic and physiologic differences between avian and mammal species and between ruminant and non-ruminant. Data have been fitted by Sigma-plot software to a potential equation: Pharmacokinetic parameter = aW^b . Results for enrofloxacin Vdss (L) were $3.78W^{0.97}$ (birds), $4.91W^{0.85}$ (mammals), $7.57W^{0.76}$ (ruminants) and $1.92W^{1.02}$ (non-ruminants), and for CL (ml/min) were $13.76W^{1.4}$ (birds), $39.34W^{0.72}$ (mammals), $27.4W^{0.84}$ (ruminants) and $2.72W^{1.11}$ (non-ruminants). For marbofloxacin: Vdss (L) were $0.98W^{1.19}$ (birds), $1.41W^{1.00}$ (mammals), $1.56W^{0.96}$ (ruminants) and $2.32W^{0.94}$ (non-ruminants), and for CL (ml/min) were $4.10W^{0.72}$ (birds), $2.32W^{1.11}$ (mammals), $1.88W^{1.16}$ (ruminants) and $5.27W^{0.97}$ (non-ruminants). From allometric equations, theoretical and real values have been compared. For enrofloxacin and marbofloxacin CL, theoretical and real values are similar for avian from avian equations. For small mammals (lower than 100 kg b.w.), mammal, ruminant or non-ruminant species produced similar results. Nevertheless in the case of large animal species (upper than 300–500 kg) specific equation predicts the best values. Similar consideration might be established for enrofloxacin Vdss, whereas marbofloxacin Vdss does not show this behavior. We can conclude that clearance is strongly influenced by anatomy and physiology in large animals for both quinolones, and it is the best pharmacokinetic parameter for allometric scaling.

P73-05

CORRELATION BETWEEN IN VIVO/IN VITRO ENROFLOXACIN CONCENTRATIONS AFTER INTRAMAMMARY ADMINISTRATION TO SHEEP

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Introduction: Enrofloxacin is an antibiotic for veterinary use only which is employed against major pathogens Gram – and Gram + in sheep. We evaluated enrofloxacin concentrations after intramammary administration in the isolated perfused sheep udder and compared these results with those found in living animals.

Material and Methods: *In vitro* model (14 healthy Assaf lactating sheep): mammary glands were taken at slaughter from sheep and perfused *in vitro* with warmed and gassed Tyrode solution. After equilibration phase, one intramammary syringe of enrofloxacin (1 g enrofloxacin/5 g ointment) was applied through the teat canal. *In vivo* model (six sheep): 1 g enrofloxacin/5 g ointment was applied through the teat canal. In both models, after 180 min, glandular tissue was sampled at constant distances vertically from the teat (2, 4, 6, 8 cm). Enrofloxacin concentrations were determined by HPLC with UV detection.

Results and Conclusions: Concentrations decreased exponentially with increasing vertical distance from the teat base in the *in vitro* model. Mean enrofloxacin concentration at 2 cm was 123.80 µg/g; at 4 cm, 54.48 µg/g; at 6 cm, 36.72 µg/g, and at 8 cm, 26.42 µg/g tissue. This behavior corresponds with that found *in vivo*. Experimental data best fit

a monoexponential model. The diffusion kinetics is similar in both models. We can predict enrofloxacin concentrations after intramammary administration *in vivo* at different sampling heights multiplying the value *in vitro* by $2.693 (C_{in\ vivo} = 2.693.C_{in\ vitro})$ or by the equation: Concentración = $478.804.e^{-0.251.height}$.

P74-05

TISSUE DISTRIBUTION OF ENROFLOXACIN AFTER INTRAMAMMARY ADMINISTRATION IN THE ISOLATED PERFUSED SHEEP UDDER

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Introduction: Enrofloxacin is a fluoroquinolone which could be effective in the treatment of mastitis, one of the most important diseases in the dairy industry. Enrofloxacin concentrations were determined in the isolated perfused sheep udder after intramammary administration, and results were compared with the minimum inhibitory concentration (MIC) established for some pathogen microorganisms causing mastitis in sheep. **Materials and Methods:** Fourteen healthy Assaf lactating sheep were used. Mammary glands were taken at slaughter from sheep and perfused *in vitro* with warmed and gassed Tyrode solution. After equilibration phase, one intramammary syringe of enrofloxacin (1 g enrofloxacin/5 g ointment) was applied through the teat canal. After 180 min, 5 g glandular tissue was sampled at constant distances vertically from the teat (2, 4, 6, 8 cm). Enrofloxacin concentrations in glandular tissue were measured by HPLC with UV detection. C_{max}/MIC_{90} ratios were calculated for *Staphylococcus aureus*, *Mycoplasma agalactiae*, *Escherichia coli*, *Streptococcus* spp., and *Pasteurella haemolytica* using mean C_{max} values obtained in each sampling.

Results and Conclusions: After intramammary administration enrofloxacin concentrations in the glandular tissue decreased exponentially with increasing vertical distance from the teat base. Tissue concentrations were higher than MIC_{90} ($C_{max}/MIC_{90} > 8$) of *Staphylococcus aureus* and *Mycoplasma agalactiae* (0.5 µg/ml), *Escherichia coli* and *Streptococcus* spp. (0.06 µg/ml), and *Pasteurella haemolytica* (0.03 µg/ml). Enrofloxacin administered at 1 g (intramammary administration) could be used for treatment of mastitis caused by these microorganisms.

P75-05

A PREDICTIVE PHARMACOKINETIC/PHARMACODYNAMIC MODEL OF FENTANYL FOR SEDATION IN INFANTS BASED ON ONTOGENY APPROACH

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Introduction: Fentanyl is used for prolonged sedation in infants. Initial doses are usually extrapolated from adults, which is questionable because age-related changes on PK/PD take place in infants (28 days–23 months). Subsequent doses are adjusted based on clinical response. Neither of these approaches ensures an adequate degree of sedation.

Aim: Develop a predictive PK/PD model of fentanyl for sedation in infants that improves dose individualization.

Materials and Methods: A physiologically based ontogenic approach was used for estimation of tricompartamental PK parameters, taking the adult as reference and considering age-related physiological changes. Volumes of distribution (V) and intercompartmental clearances (CLd) were related to body water and cardiac output, respectively.

alfa1-glicoprotein, CYP3A4 and liver blood flow were considered for systemic CL (CLs). PD was described by means of a semiparametric model, after brain perfusion dependent scaling of the effect compartment equilibrium rate constant (ke0). Simulations of fentanyl effect on different ages and dosing protocols were performed in NONMEM using estimated parameters and their interindividual variability.

Results: For a representative infant (1 year) the following parameters were obtained: 2.8, 6.0, 49.9 and 58.7 l for V1, V2, V3 and Vss; 1.2 and 0.5 l/min for CLd1 and CLd2, respectively. CLs is affected by variability, ranging from 0.17 to 0.34 l/min. These parameter values are similar to those published in the scarce literature available.

Conclusions: This physiological paediatric PK/PD model predicts fentanyl behaviour in the studied ages, allowing proposal of more efficient dosing regimens. However, plasma monitoring is highly recommended given the important variability in this subpopulation.

P76-05

PHARMACOKINETICS OF DANOFLOXACIN AFTER INTRAVENOUS, SUBCUTANEOUS AND AS A LONG-ACTING POLOXAMER 407 GEL FORMULATION TO LACTATING GOATS

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Introduction: Danofloxacin is a fluoroquinolone antimicrobial drug developed for use in veterinary medicine. Poloxamer 407 (P407) has some advantages as its low toxicity, and a high-solubilizing capacity for different drugs. The objective of the present study was to determine the pharmacokinetics of danofloxacin in goats after intravenous (IV), subcutaneous and as a long-acting poloxamer 407 gel formulation.

Materials and Methods: A cross-over study (2 × 2 × 2) was carried out in six healthy Murciano-Granadina lactating goats. Each animal received single IV injections of danofloxacin (Advocin[®] 180) at a dose of 6 mg/kg (IV, SC) and a subcutaneous long-acting P407 formulation (SC-P407) of 18 mg/kg. Plasma concentrations of danofloxacin were determined by HPLC with fluorescence detection. Blood samples were collected before and at predetermined times over a 120 h-period. Non-compartmental pharmacokinetic parameters were estimated using WinNonlin Professional[™] (version 5.0).

Results and Discussion: The danofloxacin terminal half-life (t_{1/2z}) was 2.97 ± 1.78 h after intravenous administration. The apparent volumes of distribution calculated by the area method (V_z) and at steady-state (V_{ss}) were 3.01 ± 0.98 and 1.61 ± 0.18 l/kg, respectively, indicating a wide body distribution. Total body clearance was 0.75 ± 0.10 l/kg h. After extravascular administrations, terminal half-lives were 1.50 ± 0.19 h (SC) and 8.26 ± 1.66 (P407). MRT values obtained were 4.39 ± 0.29 h (SC) and 4.42 ± 0.48 h (P407).

Conclusion: The systemic danofloxacin exposure achieved in goats following IV, SC and SC-P407 administration is consistent with the predicted blood levels needed for a positive therapeutic outcome.

P77-05

PHARMACOKINETICS OF A LONG-ACTING POLOXAMER 407 GEL FORMULATION FOR MARBOFLOXACIN IN GOATS

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Introduction: Marbofloxacin is a fluoroquinolone antimicrobial drug developed for use in veterinary medicine. Poloxamer 407 (P407) has

some advantages as its low toxicity, and a high-solubilizing capacity for different drugs. The objective of the present study was to determine the pharmacokinetics of marbofloxacin in goats after intravenous (IV), subcutaneous (SC) and as a long-acting poloxamer 407 gel formulation.

Materials and Methods: A study (n = 6) was carried out in six healthy Murciano-Granadina lactating goats. Each animal received single IV injections of danofloxacin (Marbocyl[®]10%) at a dose of 2 mg/kg (IV, SC) and a subcutaneous long-acting P407 formulation (SC-P407) of 6 mg/kg. Plasma concentrations of danofloxacin were determined by HPLC with fluorescence detection. Blood samples were collected before and at predetermined times over a 120 h-period. Non-compartmental pharmacokinetic parameters were estimated using WinNonlin Professional[™] (version 5.0).

Results and Discussion: The marbofloxacin terminal half-life (t_{1/2z}) was 0.83 ± 0.02 h after intravenous administration. The apparent volume of distribution calculated by the area method (V_z) was 0.94 ± 0.25 l/kg, indicating a wide body distribution. Total body clearance was 0.30 ± 0.07 l/kg/h. After extravascular administrations, terminal half-lives were 1.59 ± 0.01 h (SC) and 2.29 ± 0.05 (P407). MRT values obtained were 3.21 ± 1.01 h (IV), 4.53 ± 0.90 h (SC) and 7.21 ± 3.56 h (P407).

Conclusion: The systemic marbofloxacin exposure achieved in goats following IV, SC and SC-P407 administration is consistent with the predicted blood levels needed for a positive therapeutic outcome.

P78-05

TEN YEARS OF TACROLIMUS DOSE INDIVIDUALIZATION IN PATIENTS AFTER LIVER TRANSPLANTATION: PHARMACOKINETIC CONSIDERATIONS AND PATIENT PATHOPHYSIOLOGY

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Introduction: Tacrolimus (TAC) improved immunosuppression in liver transplantation (LTx). Nevertheless, interindividual differences in dose-response persist, mainly due to TAC elimination via the transplanted organ. Dose adjustment is blood level (C_{min}) based but difficulties have been reported. The aim was to compare TAC monitoring data from two LTx treatment periods at Cruces Hospital, 1998 (discovery) and 2007, when the PK peculiarities were considered.

Materials and Methods: Retrospective routine monitoring records (dose, C_{min}) and biochemistry variables, from 36 de novo LTx patients (2007), were analyzed (SPSS) and compared with data from 75 LTx patients in 1998, all under BID TAC and empirical dose adjustment. Combined analysis was done with NONMEM.

Results: Variability in C_{min}/Dose was reduced in 2007, associated with lesser dose changes (in equilibrium). The evolution of AST with TPT was bi-exponential for both years with the inflexion near 3 days, but different magnitudes: 2007 was AST = 762*exp(-0.7*TPT) + 97*exp(-0.04*TPT) and 1998 was AST = 1145*exp(-0.62*TPT) + 51*exp(-0.0052*TPT). The combined PK model was similar to that for 1998, but 'year of LTx' was a covariate for clearance. Individual means were, CL/F₀₋₃ = 11.58 l/h and CL/F₄₋₁₅ = 18.79 l/h (1998) and CL/F₀₋₃ = 13.60 l/h and CL/F₄₋₁₅ = 24.8 l/h (2007), explained by the difference in AST evolution.

Conclusion: Most of the complications with monitoring of TAC in LTx could be resolved with careful application of PK principles combined with patients' idiosyncrasies. The use of a PK model for TAC, refined in each clinical situation, could be an important tool for dose optimization early post LTx.

MOLECULAR PHARMACOLOGY

P79-06

DIFFERENCES IN THE ERK1/2 SIGNALLING PATHWAY OF $\alpha 1$ -ADRENOCEPTOR SUBTYPES

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Recent studies have shown that adrenoceptors (AR) can themselves initiate ERK cascade by both G-protein and β -arrestin-dependent process, with a subtype different regulation of ERK1/2 activation over time [1]. The aim was to analyze the temporal relationship between the activation of ERK1/2 and $\alpha 1$ -ARs trafficking patterns. HEK293 cell lines stably transfected with each $\alpha 1$ -AR subtype were stimulated with phenylephrine (100 μ M) in the presence or absence of methyl- β -cyclodextrin (10 mM), which alters membrane integrity, or LY294002 (50 μ M), an inhibitor of exocytosis and recycling of receptors. ERK1/2 activation was quantified by Western blot. In $\alpha 1A$ - and $\alpha 1B$ -AR transfected cells, phenylephrine induced an early (1–5 min) and transient ERK1/2 phosphorylation dependent on PKC [1]. This peak was inhibited in both subtypes by methyl- β -cyclodextrin, indicating its dependence on cellular membrane integrity. A later (10–15 min) sustained ERK1/2 activation, which is dependent on receptor internalization [1], was observed in $\alpha 1A$, but not in $\alpha 1B$ -ARs. Interestingly, 60 min in the presence of methyl- β -cyclodextrin seems to potentiate this late ERK phosphorylation which was unaffected by LY294002, indicating that this signal does not depend on the continuous recycling of receptors but is increased when the membrane was completely disrupted. The PKC-independent ERK phosphorylation mediated by $\alpha 1D$ -AR was inhibited by LY294002 but not by methyl- β -cyclodextrin (30 or 60 min), corroborating that this signal is not dependent on cellular membrane integrity but a recycling of this subtype is necessary to ERK activation.

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Reference:

1. Perez-Aso et al. J Biol Chem. (in review).

P80-06

FACTORS CONTRIBUTING TO HYPOXIA SECRETORY PATTERNS OF RAT EMBRYO CHROMAFFIN CELLS

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During fetal and early perinatal life, adrenal medulla chromaffin cells behave as oxygen sensors. Thus, the hypoxia-induced secretion of catecholamine (HIS) serves for adaptation, survival and to prevent irreversible brain damage. We report here striking novel aspects on the HIS response patterns in rat embryo chromaffin cells (ECCs). For instance, the distribution of large dense-cored vesicles (LDCVs) as a ring underneath the plasmalemma in ECCs, compared with a homogeneous distribution throughout the cytosol in the rat mothers chromaffin cells (MCCs). Or the smaller sizes of ready-release vesicle pool (RRP) or immediate-release vesicle pool (IRP) with smaller quantal size in ECCs, compared with MCCs. We also found that hypoxia-elicited depolarization and the HIS response of ECCs were mostly controlled by the L-subtype (α_{1d} , Cav 1.3) of voltage-activated calcium channels (VACCs); although expressed at high densities, the N-type (α_{1B} , Cav 2.2) and P/Q-type (α_{1A} , Cav 2.1) VACCs did not contribute to such control. T-type (α_{1E} , Cav 3) channels seemed to play a less clear role in controlling the HIS response. Data reveal novel aspects on the factors that regulate the HIS response in ECCs. This may have physiopathological and therapeutic importance to prevent brain damage elicited by hypoxic stress during fetal life, at birth and during perinatal life.

P81-06

BLOQUERS OF T-TYPE Ca^{2+} CHANNELS ALSO INHIBIT HIGH VOLTAGE ACTIVATED CALCIUM CURRENTS

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The Ni^{2+} and mibefradil are ligands used to characterize low threshold voltage-dependent Ca^{2+} channels (VDCCs), also called T-type. However, some concentrations of these drugs, commonly used in the literature were found to be unspecific blockers of T-type currents. We studied their effects on Ba^{2+} currents (IBa), catecholamine release and cytosolic Ca^{2+} transients ($[Ca^{2+}]_c$) in a primary culture of adrenal chromaffin cells (CC) from three different animal species, rat embryo (RECC), mouse (MCC) and bovine chromaffin cells (BCC). BCC express only the high threshold VDCCs, however RECC express both T-type and high threshold VDCCs. This study was performed by using patch-clamp technique, amperometry and fluorescence microscopy. Ni^{2+} blocked, in a concentration-dependent manner, IBa (5 mM), elicited by a 50 ms depolarizing pulses (DP). Furthermore cytosolic calcium transient peak, in BCC, was reduced with an IC50 of $(4.27 \times 10^{-5} \pm 0.04)$ M ($3.59 \times 10^{-5} \pm 0.07$) M and $(6.91 \times 10^{-6} \pm 0.37)$ M after 1, 4 and 7 min of perfusion of Ni^{2+} . Mibefradil blocked in a concentration-dependent manner $[Ca^{2+}]_c$ in CCB with an IC50 of $(1.10 \times 10^{-7} \pm 0.21)$ M, $(4.87 \times 10^{-8} \pm 0.44)$ M, $(8.77 \times 10^{-8} \pm 0.13)$ M after 1, 4 and 7 min of perfusion respectively. Mibefradil blocked in BCC the IBa with an IC50 of (4.00 ± 0.31) μ M and (6.38 ± 0.66) μ M in RECC.

P82-06

NOVEL *IN VITRO* PHARMACOLOGICAL BEHAVIOUR OF COMPOUNDS ACTIVE AT SEROTONIN 5-HT7 RECEPTORS STABLY EXPRESSED IN HEK293 CELLS

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The 5-HT7 receptors are the last identified members of the serotonin receptor family. They are G protein (Gs)-coupled receptors expressed in central (CNS) and peripheral (PNS) nervous system and in the periphery. The 5-HT7 receptors have been implicated in diverse CNS functions, including circadian rhythm, sleep, depression, thermoregulation, anxiety, schizophrenia, nociception, epilepsy, and memory. Recent studies unveiled novel concepts in 5-HT7 receptor signalling, namely biphasic signalling and G protein-independent signalling, which have been related to receptor dimerization. Our aim was to perform a detailed *in vitro* pharmacological characterization of novel compounds active at 5-HT7 receptors. The *in vitro* pharmacology of two selected compounds was investigated in HEK293 cells stably expressing human or rat 5-HT7 receptors. Radioligand ($[3H]$ -LSD and $[3H]$ -SB269970) binding competition assays were performed both in cell membranes and intact cells. The efficacy of the compounds at 5-HT7-mediated or forskolin-stimulated cAMP signalling was measured in these cell lines by ELISA. The two compounds assayed fully displaced the specific binding of $[3H]$ -LSD and $[3H]$ -SB269970 in 5-HT7-expressing cell membranes, with K_i values in the nanomolar range. Concentration-response curves revealed an antagonist behaviour of the compounds at the 5-HT7-mediated cAMP pathway, while Schild analysis of 5-CT-stimulated cAMP formation in the absence or presence of increasing concentrations of the compounds showed an insurmountable antagonism for both compounds. In conclusion, the two compounds studied showed an *in vitro* pharmacological behaviour in the line of recent reported models of 5-HT7-mediated inactivation of cAMP signalling by classical 5-HT7 antagonist ligands as risperidone.

HORMONAL PHARMACOLOGY AND METABOLISM

P83-07

MECHANISMS IMPLICATED IN THE EFFECTS OF PROPIONYL-L-CARNITINE ON THE OBESITY-RELATED INSULIN RESISTANCE STATE

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Chronic obesity is frequently related to insulin resistance. Propionyl-L-carnitine (PLC) is an endogenous non-protein amino acid that plays an important role in glucose and lipid metabolism. We previously reported that PLC ameliorated the insulin-resistant state in obese animals. Here, we evaluated the mechanisms by which PLC may exert such effects. C57BL/6J mice were fed a high fat (HF) diet (42% from total energy) during 9 weeks. Next, two different protocols were executed: (i) Hearts were perfused in a retrograde Langerdorff system. PLC was load (1 mM, 60 min) and the rate of ¹⁴C-glucose and ³H-palmitate oxidation was measured in the presence and the absence of insulin. The activities of pyruvate dehydrogenase (PDH) and carnitine acetyltransferase (CAT) were also determined. (ii) Mice were HF-fed 4 additional weeks and divided into two groups, one receiving PLC (200 mg/kg) in the drinking water and a control group receiving PLC-free water. Total L-carnitine and acylcarnitines tissue levels, mitochondrial activity and in situ nitric oxide production were measured in heart or skeletal muscle. HF-fed animals showed a deficit of total tissue L-carnitine, associated to lower basal and insulin-stimulated glucose oxidation rates and greater accumulation of long-chain acylcarnitines. PLC treatment improved the insulin-induced response in the myocardial metabolism, without altering PDH or CAT. PLC did not change mitochondrial activity, but restored total L-carnitine, diminished long-chain acylcarnitines and enhanced NO production. In conclusion, PLC improved insulin-induced response by restoring L-carnitine tissue levels and diminishing potentially toxic intermediates from incomplete lipid metabolism in insulin-resistant obese animals.

P84-07

IMPAIRED INSULIN STIMULATED NITRIC OXIDE PRODUCTION INDUCED BY HYPERGLUCEMIA IN HUMAN ENDOTHELIAL CELLS IS IMPROVED BY PPAR-B ACTIVATION

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It has been described that peroxisome proliferator-activated receptor beta/delta (PPAR-beta) activation improves insulin sensitivity in diabetic db/db. In this study we examine the possible protective effects of the agonists of PPAR-beta, GW0742 and L165041, on impaired insulin signaling found in high glucose cultured primary human umbilical vein endothelial cells (HUVEC). HUVEC were cultured with GW0742 or L165041 (1 or 10 μ M) during 24 h in low (5 mM) or high glucose (30 mM). Cells were then used to measure nitric oxide (NO) production by DAF fluorescence, or by test both Akt and eNOS phosphorylation by western blots, under basal and insulin (100 nM for 30 min) stimulated conditions. HUVECs incubated in high glucose medium reduced significantly the insulin-dependent production of NO and (Ser473) Akt and (Ser1177) eNOS phosphorylation as compared to cells exposed to low glucose. The co-incubation with both PPARs agonists increased the NO production stimu-

lated by insulin in high glucose cultured cells, being without effect in cells incubated in low glucose. This improvement in endothelial function was accompanied by increased Akt and eNOS phosphorylation. Both functional and expressional effects induced by both agonists were suppressed when HUVEC were also incubated with the PPAR-beta antagonist GSK0660 (1 μ M) and the pyruvate dehydrogenase kinase (PDK)-4 inhibitor dichloroacetate (2 mM). Our results suggest that PPAR-beta activation improves Akt-eNOS pathway, leading to increase NO production stimulated by insulin in high glucose incubated HUVECs, at least in part, through PDK4 activation.

P85-07

ADRENOCORTICAL PYRROLIDON CARBOXYPEPTIDASE ACTIVITY IS RELATED TO DOXAZOSIN-INDUCED DECREASE OF CORTISOL LEVELS IN FEMALE BUT NOT MALE WISTAR RATS

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Introduction: It has been described the inhibitory effects of alpha-1 adrenoceptor antagonists on cortisol release by interfering with enzymes of the hormone biogenesis pathway. It has been also described the influence of sex hormones in the physiology of the HPA axis through the modulation of the pyrrolidone carboxypeptidase type I (PcpI) and type II (PcpII) activities. Here we analyze the effects of doxazosin on PcpI and PcpII specific activities in the synthesis of cortisol in male and female Wistar rats, to determine the existence of gender differences.

Material and Methods: Thirty male and thirty female Wistar rats were used and randomly divided into male and female control and doxazosin groups treated subcutaneously with the vehicle or 10 mg/kg doxazosin during 15 days. Serum and adrenal cortex were obtained and the last processed to obtain both soluble and membrane-bound fractions. Serum cortisol was measured by FPIA. Adrenocortical PcpI and PcpII activities were assayed fluorometrically.

Results: Control female rats showed significantly higher levels of cortisol than males. We did not found differences in cortisol levels in males after doxazosin treatment. On the contrary, in females, doxazosin treatment significantly decreased circulating levels of cortisol. No significant differences were found in membrane-bound PcpII activity due to doxazosin treatment or gender. However, soluble PcpI increases significantly in females after doxazosin treatment, whereas no changes were observed in males.

Conclusions: We conclude that PcpI specific activity is involved in the alpha-1-adrenergic receptor blockade-induction of cortisol release through pyroglutamyl-ended peptides which act in the adrenal gland of female but not male Wistar rats.

P86-07

MORPHOLOGICAL AND FUNCTIONAL DISTURBANCES IN PANCREATIC B-CELLS AND ADIPOCYTES FROM IRS-2 DEFICIENT MICE

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The development of diabetes in *Irs2*^{-/-} null mice has been well characterized and is attributed to β -cell deficiency and severe insulin resistance. The aim of the present study is to analyze differences in β -cell morphology and functional response to hypoglycemic drugs in both pancreatic

islets and fat-cells obtained from IRS-2 deficient mice. The β -cell size and density were analyzed in insulin-stained pancreas sections from IRS-2 deficient mice. The study of fat-cell and β -cell function was carried out by measuring lipolysis and insulin secretion respectively in those cells. Morphometric and densitometric analysis revealed that reduction in β -cell mass produced by the absence of IRS-2 signals was less severe in female than in male KOs (25% vs. 43%). Galanine (neuropeptide related with autonomic nerves) inhibited less efficiently insulin secretion induced by glucose in islets from male Irs-2 than in WT control (35% vs. 65%). These confirming previous experiments performed with alpha-adrenergic agonists. Sulfonylureas (tolbutamide and glibenclamide, K_{ATP} channel inhibitors) did not modify insulin secretion, and neither diazoxide, a potassium channel opener, changed that parameter. Furthermore, nimodipine (calcium channel blocker) induced less inhibition on insulin secretion in IRS-2 mice (40%) vs. WT control (85%). Another hypoglycaemic drug, rosiglitazone (ppary agonist) did not modify insulin secretion or basal lipolysis. These results confirm that, in addition to the morphological differences showed between sexes in mice IRS-2 deficient, there are also dysregulation of sympathetic activity and alterations in calcium channels. These changes would be involved in the pathogenesis of type-2 diabetes.

P87-07

IN VITRO EFFECTS ON LIPOLYTIC ACTIVITY AND INSULIN SECRETION OF ANTIPSYCHOTICS AFTER ITS CHRONIC ADMINISTRATION IN A SCHIZOPHRENIC RAT MODEL

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Glucose intolerance and/or insulin resistance are associated with the administration of some antipsychotics. Risperidone, an atypical antipsychotic, is prescribed widely and fortunately this drug has shown less risk of metabolic disturbances. Our aim was to know the chronic effects of risperidone on glucose homeostasis, by analyzing lipolysis and insulin release on a rat model accepted for the study of schizophrenia. Wistar male rats, with neonatal lesion in the ventral hippocampus (NLVH) performed by stereotaxic injection of ibotenic acid at 7th day post-birth, were used as the schizophrenic animal model. Following, *in vitro* effects of risperidone (2 mg/kg/day, orally for 2 months) was studied on 75 \pm 5 day-old rats (healthy and NLVH). Afterwards, lipolytic activity (basal and evoked) was measured on perirenal fat cells; insulin release was studied in isolated islets. For comparisons, responses to haloperidol (as typical antipsychotic) were also tested. The basal lipolysis after chronic administration of risperidone was significantly diminished in NLVH rats in contrast to the control group. The lipolytic response induced by either isoproterenol or forskolin was similar. Compared with controls, the insulin release was slightly increased in the NLVH group but results were not significant. The reduction of lipolytic activity by risperidone may block the release of non-esterified fatty acids from adipocytes, thereby diminishing the lipotoxicity on islet beta cells, and these effects may explain the lower tendency to glucose intolerance or metabolic syndrome associated to this drug.

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P88-07

IDENTIFICATION OF ACTIVE COMPOUNDS AT DOR (DIABETES AND OBESITY REGULATED) AS PUTATIVE ANTI-OBESITY/ANTIDIABETIC DRUGS BY HIGH THROUGHPUT SCREENING

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The high incidence of obesity in developed countries as well its association with the insulin resistance syndrome and the non-insulin dependent diabetes mellitus, has led to a significant increase in the studies devoted to a better comprehension of diabetes and its therapeutics. Among these studies it has been reported a gene which is diabetes and obesity regulated (DOR), being the expression of the related protein in diabetic and obese rats [1,2]. Our aim in this work was to develop a High Throughput Screening (HTS) methodology which allows detecting compounds increasing the expression of this protein. The methodology developed was based in luciferase reporter gene assays and showed to be robust with *Z'* values over 0.5 and a signal/background ratio over 2. The Prestwick[®] chemical library was screened being eight ligands identified as hits. Concentration-response curves of the eight hits were constructed showing one of them an EC50 value of 25.4 nM and an efficacy of 176% when compared with maximal DOR expression increase induced by T3. In summary, in the present work we have detected by HTS a low-molecular weight compound which increases DOR expression being promising starting point for future development in order to obtain a pre-clinical candidate for treating obesity and/or diabetes.

This work was supported by DioMed project from SUDOE-Interreg program (SOE1/P1/E178). JB is recipient of an Isabel Barreto contracto from Xunta de Galicia.

References:

1. Baumgartner et al. Plos One 2007;11:e1183.
2. Mauvezin et al. EMBO Reports 2010;37-44.

P89-07

DEVELOPMENT OF AN AUTOMATED METHODOLOGY FOR MEASURING NATIVE GLP-1R ACTIVATION USEFUL FOR THE SCREENING OF COMPOUNDS WITH PUTATIVE ANTI-DIABETIC ACTIVITIES

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The pathophysiology of type 2 diabetes mellitus consists mainly in insulin resistance and β -cell dysfunction. Interest in gastrointestinal incretins in the possible treatment of this disease has been revitalized with the introduction of glucagon-like peptide-1 (GLP-1) therapy. GLP-1 increases cAMP levels by activating a specific G protein-coupled receptor (GLP-1R). Although unable to elicit insulin secretion on its own, GLP-1-induced cAMP potentiates glucose-induced insulin secretion (Holz et al. J Biol Chem 1995;270:17749-17757). GLP-1 is rapidly degraded by dipeptidyl peptidase IV (DPP-4) thus, current strategies are focused in the use of GLP-1 and DPP-4 inhibitors (i.e. vildagliptin), and the development of DPP-4 resistant GLP-1 agonists (i.e. Exendin-4). The screening of compounds over native GLP-1 receptors endogenously expressed in a rat insulinoma cell line would lead to the hit identification in a more physiological environment. Therefore, our aim in the present study was to develop a methodology for HTS on GLP-1R receptor, by measuring the cAMP produced by INS-1E cells, which would enable the identification of compounds with potential antidiabetic activity. Using an immunoassay of competition between cAMP produced by INS-1E cells and cAMP labeled with a fluorophore, we evaluate different conditions by modifying parameters such as the presence of FBS, the use of DPP-4

inhibitors, the concentration of glucose, etc. Exendin-4 evaluated by this method showed a potency ($EC_{50} = 98 \text{ pM}$) analogous to that described (33 pM Thorens et al. Diabetes 1993;42:1678–1682). In summary, we developed a robust and reproducible methodology to evaluate the activation of endogenous GLP-1R on a rat insulinoma cell line.

P90-027

IDENTIFICATION OF NOVEL SPECIES-SELECTIVE LIGANDS OF THE G PROTEIN-COUPLED RECEPTOR GPR35

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GPR35 is a poorly characterized GPCR (G protein-coupled receptor) that has been suggested to be a potential exploratory target for conditions ranging from diabetes to inflammation, asthma and hypertension (Biochem J 2010;432:451–459). GPR35 remains potentially an orphan receptor, although both kynurenic acid and lysophosphatidic acid were reported to activate GPR35 but this interaction is not at physiological concentrations. The best characterized synthetic GPR35 agonist, zaprinast, displays considerable species orthologue selectivity. A major impediment to target validation and further study of the role of the receptor has been a paucity of pharmacological tools to study GPR35. HTS of chemical library would lead to the identification of both agonist and antagonist ligands for GPR35. We carried out a BRET GPR35- β -arrestin-2 interaction assay with both human and rat orthologues of GPR35 screening the entire Prestwick Chemical Library[®] at 10 μM in 96-well plate format. We identified nine compounds possessing agonist activity including the previously described ligand zaprinast. Although a number of active compounds, including cromolyn disodium and dicumarol, displayed similar potency at both orthologues of GPR35, a number of ligands, including pamoate and niflumic acid, had detectable activity only at human GPR35 whereas others, including zaprinast and luteolin, were markedly selective for the rat orthologue. In summary, the ligands reported in this study add substantially to the available pharmacology of GPR35 and provide tool reagents to further probe its function and also underlie the importance of understanding the basis of orthologue selectivity.

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P91-07

OLEIC ACID INHIBITS THE ENDOPLASMIC RETICULUM STRESS INDUCED BY THE SATURATED FATTY ACID PALMITATE IN SKELETAL MUSCLE CELLS THROUGH AMPK ACTIVATION

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Enhancement of saturated fatty acids levels in plasma is related with inflammation and insulin resistance in skeletal muscle. In contrast, mono-unsaturated fatty acids, such as oleate, may prevent these processes, although the molecular mechanisms are poorly understood. Given that endoplasmic reticulum(ER) stress is associated with inflammation and

insulin resistance, we evaluated whether oleate is able to prevent the ER stress induced by palmitate in skeletal muscle cells. Skeletal muscle cells of mouse and human origin were incubated for 16 h with palmitate (0.5 mM), oleate (0.5 mM) or palmitate (0.5 mM) and oleate (0.3 mM) in the presence or absence of GW6471 (10 μM), GSK0660 (1 μM), or compound C (30 μM). Exposure of mouse skeletal muscle cells to palmitate increased the mRNA levels of endoplasmic reticulum stress markers (ATF3, BIP, CHOP, Hsp70 and sXBP1), as well as the protein levels of phosphorylated IRE1 α . Palmitate also induced NF- κB DNA-binding activity and, consequently, IL-6 expression and secretion. Interestingly, these changes were not observed in cells exposed to oleate. Furthermore, oleate prevented the ER stress induced by palmitate. Similar results were observed in human skeletal muscle cells. Finally, coincubation with compound C, GW6471 and GSK0660 demonstrated that oleate prevented the ER stress induced by palmitate through AMPK activation, while PPAR α and PPAR β/δ were not involved in this effect. Our results suggest that oleate may prevent palmitate-induced inflammation and insulin resistance in human and mouse skeletal muscle cells by means of ER stress inhibition. AMPK activation by oleate appears to be involved in this inhibition.

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P92-07

OBESITY-DEPENDENT CANNABINOID 1 MODULATION OF PROLIFERATION IN ADULT NEUROGENIC REGIONS

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Endocannabinoid signaling participates in the control of neurogenesis, especially after brain insults. Among them, obesity could be the cause of alterations in physiology affecting neurogenesis; however it is unclear whether cannabinoid signaling may modulate neural proliferation in obese animals. We analyzed the impact of obesity defined by two different approaches, a high-fat diet (HFD, 60% fat) and a standard/low fat diet (STD, 10% fat), and the response to a subchronic treatment with CB1 receptor inverse agonist AM251 (3 mg/kg) on cell proliferation of two relevant neurogenic regions: the subventricular zone in the striatal wall of the lateral ventricle (SVZ) and the subgranular zone of the dentate gyrus (SGZ); and also in the hypothalamus due to its relevant role in energy metabolism. We found evidence of an interaction between the diet-induced obesity and CB1 signaling in the regulation of cell proliferation. Our results showed that AM251 reduced caloric intake and body weight in obese rats, as well as corrected plasma levels of cholesterol and triglycerides. We described for the first time that AM251 can modulate cell proliferation in HFD-obese rats only. We observed an increase in the number of 5-bromo-2-deoxyuridine-labelled (BrdU+) cells in the SGZ, but a decrease in the number of BrdU+ cells in the SVZ and the hypothalamus of AM251-treated HFD rats. These BrdU+ cells expressed the neuron specific β III-tubulin. These results suggest that obesity may impact cell proliferation in the brain selectively, and provide support for a role of CB1 signaling regulation of neurogenesis in response to obesity.

GASTROINTESTINAL PHARMACOLOGY

P93-08

PROTECTIVE EFFECT OF AN ELLAGIC ACID ENRICHED-POMEGRANATE EXTRACT, A NATURAL POLYPHENOLIC-RICH FRUIT, IN A MURINE MODEL OF CROHN'S DISEASE

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Introduction: A complex system of intracellular signaling molecules, such as mitogen-activated protein kinases (MAPKs) or the NF- κ B nuclear transcription, influences the uncontrolled immune system activation in Crohn's disease (CD). In previous studies, *Punica granatum* L. (pomegranate) has been shown to exert antioxidant and anti-inflammatory effects. Besides, we have documented that Ellagic Acid (EA), a natural polyphenol compound present in pomegranate, decreased the degree of inflammation associated with acute colonic inflammation.

Aim: Study the effects of a dietary EA-enriched pomegranate extract (PE) in a murine chronic model of CD.

Materials and Methods: Colonic injury was induced by intracolonic instillation of trinitrobenzenesulphonic acid (TNBS). Rats were fed with diets : (i) standard diet, (ii) pomegranate extract (PE) 0,6%, (iii) EA 0,02% and (v) EA 0,02% enriched- PE 0,6% diets during 30 days before TNBS instillation and during 2 weeks before killing. Inflammation response was assessed by MPO activity and TNF- α production. iNOS, COX- 2, p38, JNK, p-ERK1/2 MAPKs, IKB α inhibitory protein, and nuclear p65 NF- κ B expressions were studied by western blotting in colon mucosa.

Results: There was a significant reduction in the severity of colonic damage after dietary intervention. Also MPO activity and the TNF- α levels were significantly reduced in dietary fed rats after TNBS administration. Diets drastically decreased COX-2 and iNOS overexpression, reduced the activation of MAPKs and prevented the degradation of the inhibitory protein IKB- α and decreased nuclear p65 NF- κ B expression.

Conclusions: PE and EA diets reduce the damage in a rat model of CD, alleviating the oxidative events and returning pro-inflammatory proteins expression to basal levels probably through MAPKs and NF- κ B signaling pathways.

P94-08

THE VIABILITY OF LACTOBACILLUS FERMENTUM CECT5716 IS NOT REQUIRED FOR ITS INTESTINAL ANTI-INFLAMMATORY ACTIVITY IN THE TNBS MODEL OF RAT COLITIS

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Introduction: The probiotic *Lactobacillus fermentum* shows intestinal anti-inflammatory properties in experimental colitis. The present study evaluated if probiotic viability was necessary to obtain beneficial effects in intestinal inflammation. Finally we studied its activity on epithelial cell activity.

Materials and Methods: *Lactobacillus fermentum* CECT5716 (Biosearch, S.A.) was given orally (5×10^8 CFU/rat/day) to Wistar rats, live

or dead (95°C for 30 min), for 2 weeks before TNBS colitis induction, and there after until colonic evaluation 1 week later, both macroscopically and biochemically: myeloperoxidase activity (MPO), glutathione content (GSH), TNF- α and IL-1 β levels, as well as iNOS expression. *In vitro* experiments in Caco-2 cells to evaluate the epithelial regeneration in a model of wound healing and the inhibition of IL-8 release after cell activation with IL-1 β or enterotoxigenic *Escherichia coli*.

Results: Both live and dead *Lactobacillus fermentum* showed similar anti-inflammatory effect, as evidenced by an amelioration of the damaged colon and decreased MPO. These effects were associated with a reduction in TNF- α and IL-1 β levels, increased GSH content and reduced iNOS expression. *In vitro* assays showed that this probiotic facilitated the healing of the Caco-2 monolayer (percentage of regeneration of $32.9 \pm 10.2\%$ vs. $10.4 \pm 5.1\%$ in untreated cells ($P < 0.05$)). The incubation of Caco-2 with the probiotic significantly inhibited the increased production of IL-8 induced by both IL-1 β and enterotoxigenic *Escherichia coli* ($P < 0.01$).

Conclusion: The viability of *Lactobacillus fermentum* CECT5713 did not seem to be essential for its intestinal anti-inflammatory activity in TNBS-induced rat colitis. This probiotic facilitated intestinal damage recovery and inhibited the stimulation IL-8 production.

P95-08

INTESTINAL ANTI-INFLAMMATORY EFFECTS OF THE EXTRACT AMANDA[®] IN THE TNBS MODEL OF RAT COLITIS

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Introduction: Different plant extracts have been traditionally used in human inflammatory conditions. These extracts contain antioxidants polyphenols, which contribute to these beneficial effects. Intestinal inflammation is associated with oxidative stress, and the use of these extracts-enriched polyphenols may be interesting in these intestinal conditions. We evaluated the intestinal anti-inflammatory properties of the extract Amanda[®] (30% polyphenols), in the trinitrobenzenesulphonic acid (TNBS) model of rat colitis.

Materials and Methods: Female Wistar rats (200 \pm 10 g) were assigned to seven groups (n = 8): non-colitic group, control colitic group (without treatment) and treated colitic groups: four with Amanda[®] extract (10, 25, 50 and 100 mg/kg/day) (Biosearch S.A) and one received sulphasalazine (SAZ) (100 mg/kg/day), starting the same day of TNBS colitis induction. Rats were sacrificed 1 week after, and colonic damage assessed macroscopically and biochemically: colonic myeloperoxidase activity (MPO) and total glutathione content (GSH), as well as the expression of IL-1 β , IL-6, MUC-2, MUC-3 and TFF-3 (RT-PCR).

Results: Amanda[®] extract exerted intestinal anti-inflammatory effect, evidenced by a reduction in the damage extension. This was associated to a decreased colonic MPO activity and improvement of the altered oxidative status, by attenuating the glutathione depletion observed in control colitic rats. In addition, this extract reduced the expression of IL-1 β , IL-6 and increased that of MUC-2, MUC-3 and TFF-3. The beneficial effect was higher than that obtained with SAZ, which was used as control.

Conclusion: The Amanda[®] extract showed intestinal anti-inflammatory activity in the TNBS model of rat colitis, in which the antioxidant properties play a role.

P96-08

THE SUPPLEMENTATION WITH THE PROBIOTIC *ESCHERICHIA COLI* NISSLE 1917 IMPROVES THE COLONIC RECOVERY EXERTED BY MINOCYCLINE IN A MODEL OF REACTIVATED COL

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Introduction: Antibiotics have been empirically used for human inflammatory bowel disease (IBD), being limited to short periods. Probiotics are able to attenuate intestinal inflammation due to its immunomodulatory properties, being considered as safe when chronically administered. Our aim was to test the association of minocycline (MNC), a tetracycline with immunomodulatory properties, and *Escherichia coli* Nissle 1917 (EcN) in a mouse model of reactivated colitis.

Materials and Methods: Female C57BL/6J mice were randomly assigned to different groups: Non-colitic and DSS-control groups (with-

out treatment), MNC (50 mg/kg/day p.o.), EcN (5×10^8 CFU/day; p.o.), and MNC plus EcN treated groups. Colitis was induced by adding DSS in the drinking water (3%) for 5 days; 2 weeks later, colitis was reactivated by subsequent exposure to DSS. The inflammatory status was evaluated daily by a disease activity index (DAI), and colonic damage was assessed histologically (macroscopically and microscopically), and biochemically (evaluating mRNA relative expression of different mediators by qPCR). Finally, a microbiological analysis of the colonic contents was performed.

Results: MNC and EcN exerted intestinal anti-inflammatory effect and attenuated the reactivation of the colitis, as shown by the reduced DAI values, being these effects greater when combining both treatments. This was evidenced histologically and biochemically (reduced expression of TNF- α , IL-1 β , iNOS, MMP-9 and MIP-2 together with increased MUC-3, ZO-1 and occlusion expression). Finally, the altered microbiota composition of colitic mice was partially restored after the different treatments.

Conclusion: EcN probiotic supplementation to minocycline treatment improves the recovery of the intestinal damage and prevents the reactivation of experimental colitis.

ONCOLOGIC

P97-09

NEW FLAVONE METHYL ESTERS-INDUCED CELL DEATH IN HUMAN LEUKEMIA CELLS IS AMPLIFIED BY INHIBITION OF ERK 1/2 SIGNALING

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Flavonoids are naturally occurring polyphenolic compounds which display a vast array of biological activities and are among the most promising anticancer agents. We have synthesized and analyzed the cytotoxicity of two flavonoids containing a methyl ester group in the human leukemia HL-60 and in the mitoxantrone resistant HL-60/MX1 cell lines. These compounds were designed to evaluate the influence of the introduction of a chlorine or a weak electron-donating methyl group on position 2' of the B ring (2-phenyl group). Although both compounds displayed cytotoxic activities on HL-60 cells determined by the MTT assay, the compound containing the methyl group was more cytotoxic than the compound containing chlorine against the mitoxantrone resistant HL-60/MX1. Both compounds arrested HL-60 cells at G1 phase of the cell cycle, which was associated with the accumulation of cyclin D1 and cyclin-dependent kinase inhibitor p21Cip1. The cytotoxic effects were accompanied by the concentration- and time-dependent of DNA fragmentation, increase in the percentage of sub-G1 cells and poly(ADP-ribose)polymerase cleavage. Cell death was almost completely blocked by the pan-caspase inhibitor z-VAD-fmk, and reduced by the selective caspase inhibitors z-LEHD-fmk and z-IETD-fmk. Flavone methyl esters-induced cell death was found to be: (i) associated with the release of cytochrome c and (ii) amplified by inhibition of extracellular signal-regulated kinases (ERKs) 1/2 signaling. The findings of this study suggest that these compounds may be of benefit in the development of strategies against cancer.

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P98-09

CONTINUOUS EXPOSURE TO LIGHT INCREASES OXIDATIVE STRESS AND ADRIAMYCIN-INDUCED NEPHROPATHY

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Introduction: Previous studies verified by our research group showing the effect benefits of melatonin (MEL) on the intensity of oxidative stress and biochemical expression of nephropathy caused by adriamycin (AD). This communication is focused, given the close relationship between light and fall of pineal MEL synthesis, the effect of continuous exposure of light on the degree of oxidative stress and adriamycin nephropathy.

Materials and Methods: Experimental groups: Control, AD (two consecutive doses ip: 10 mg/kg body weight) and AD + L (24 h light). Were applied as markers of oxidative stress, MDA, GSH (plasma and kidney tissue). As biochemicals: urea and creatinine (plasma) and protein and microalbumin in urine. In all groups also were estimated plasma levels of MEL.

Results and Comments: AD determines sharp increases in MDA in renal tissue and plasma and descents, equally significant, both GSH in biological media. AD also increased the levels of urea and creatinine and increased urinary excretion of protein and microalbumin. All this picture is enhanced after exposure to constant light. The significant decrease in MEL detected in these experimental conditions suggests the extraordinary antioxidant effect of endogenous MEL.

PHARMACOGENETICS AND PHARMACOGENOMICS

P99-10

INTUITIVE PHARMACOGENETICS: SPONTANEOUS RISPERIDONE DOSAGE IS RELATED TO CYP2D6, CYP3A5 AND ABCB1 GENOTYPES

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Introduction: One of the most important issues regarding the variability of effect of antipsychotic drugs is the consequences of genetic variation in regulators of drug metabolism. According to our hypotheses, in an intuitive pharmacogenetic exercise, clinicians (who are unaware of the patient's metabolic genotype) modify antipsychotic drug dosage via a trial and error strategy, in order to obtain the safest and most efficient treatment, and this will correspond with the patient's metabolic status. The aim of the present study is to evaluate whether the quantitative prescription of risperidone (dosage) is related to the patient's metabolic status determined a posteriori and blinded to the clinicians.

Patients and Methods: This prospective and observational study includes a cohort of 151 Caucasian psychiatric patients treated with risperidone. Metabolic status was defined in terms of the most relevant polymorphisms of CYP2D6 (*3, *4, *5, *6 and *1xN), CYP3A5 (*3A) and ABCB1 (G2677T).

Results: Significant differences among (Kruskal–Wallis test $P = 0.01$) and correlation with (Spearman's $r = 1$, $P = 0.02$) the dosage administered to the different CYP2D6 groups were observed, with poor metabolizers receiving the lowest doses and ultra-rapid metabolizers the highest. Regarding CYP3A5 and ABCB1 (G2677T), a trend was observed that proved not to be significant after statistical analysis.

Conclusion: We find evidence that, despite not knowing patient's metabolic status, clinicians modify risperidone dosage accordingly in order to obtain the best therapeutic option. The dosage arrived at spontaneously by clinicians could be more accurate if previous genotyping were performed. This work has been published in *Pharmacogenomics J* (2011) DOI: 10.1038/tpj.2010.91.

P100-10

THE INFLUENCE OF AROMATIC SUBSTITUENTS IN THE GLUTATHIONE S-TRANSFERASE M1 AND T1 DETOXYFYING PROCESS AND THE RISK OF DEVELOPING DRUG-INDUCED LIVER INJURY (DILI)

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Glutathione S-transferase (GST) utilizes glutathione to neutralize electrophilic metabolites, such as drugs. The genes coding for the cytosolic GSTM1 and T1 isoenzymes are polymorphic, which may lead to reduce detoxification of electrophilic compounds, as seen in carriers of the combined null genotype. The nature of aromatic ring substituents could interfere with GST substrate affinity. We aimed to analyze whether DILI patients with the GSTM1/T1 null genotype have a higher risk of hepatotoxicity when exposed to drugs with different aromatic ring substituent in the chemical structure, reducing the detoxifying properties. One hundred fifty four diagnosed DILI patients and 250 healthy controls were analyzed. Genotyping of *GSTM1* and *GSTT1* was performed using a multiplex PCR assay. The null GST M1/T1 genotype was significantly associated with DILI in patients exposed to drugs with aromatic rings ($P_c = 0.004$; $OR = 3.0$). The presence of electron donor substituents solely in the aromatic ring enhanced the risk of DILI 3.1 times ($n = 54$; $P_c = 0.019$). Meanwhile only two patients developed DILI due to drugs with electron withdrawing substituents solely in the ring. The genotype distribution did not show statistical differences when electron donor and withdrawing substituents are present at the same time in the molecule ($n = 18$; $P_c = 1.000$). Patients homozygous for the *GSTM1/T1* null genotype are at increased risk of developing DILI when exposed to drugs containing aromatic rings, particularly if the aromatic ring has electron donor substituents solely in the drug molecule.

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CLINICAL PHARMACOLOGY, SIDE EFFECTS AND TOXICOLOGY

P101-11

OXIDATIVE STRESS IN PATIENTS WITH CHRONIC KIDNEY DISEASE, SUPPLEMENTED WITH FERRIC CARBOXIMALTOSE

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Introduction: Most patients with chronic kidney disease (CKD) need a supplementation of intravenous iron that increases free iron in plasma and could increase the oxidative stress. Ferric Carboximaltose (FC; Ferinject[®]) is an iron complex that liberates iron inside of reticuloendothelial cells and does not liberate iron in plasma. Oxidative stress is increased in pre-dialysis and dialysis CKD patients, but there are not studies evaluating the influence of the treatment with FC in oxidative stress and endothelial dysfunction markers.

Materials and Methods: CKD patients with anaemia (Hb < 11 g/dl) and iron deficiency (ferritin < 100 ng/ml and/or IST < 20%) were recruited at the Nephrology department (Hospital Universitari Joan XXIII, Tarragona) (n = 36; women: 58.3%; age: 73.53 ± 10.49). They received a single dose of 15 mg/kg of FC in saline serum administrated by intravenous bolus during 30 min. Blood samples were used to determine oxidative stress biomarkers (carbonyl groups, oxidized LDL, glutathione, CAT, SOD, GPx, ORAC, FRAP) and endothelial dysfunction biomarkers (sICAM-1, sVCAM-1), before the administration of FC and 1 h, 3 weeks and 3 months later.

Results and Conclusion: Prooxidant effects of the FC administration: decreased GPx (60 min, 3 weeks and 3 months), ORACTCA (3 weeks and 3 months), and FRAP (3 months) and increased protein damage (3 months) and oxidized LDL (60 min, 3 weeks and 3 months). Improved biomarkers: increased ORAC (3 weeks and 3 months) and glutathione (3 weeks and 3 months). In conclusion, even though the glutathione system had improved, patients with CKD increased oxidative stress after 3 months of administration of FC in a single dose. However, endothelial function was not affected by treatment.

P102-11

THE TREATMENT OF PATIENTS WITH ASTHMA IN A HEALTH CENTER

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Introduction: Asthma treatment is complex. It requires a continuous monitoring of the patient to assess the grade of control and an effective use of the inhalers.

Aim: Describe the treatment of patients with asthma in a health care center.

Material and Method: It was a Transversal descriptive study. Location: Urban health care center. Study population: 583 patients with asthma (A) which 48 were selected by a systematic random sampling. The variables were age, sex, asthma classification, previous spirometry, health education on inhalator uses and prescribed treatment. Statistical analysis. It was a Descriptive statistic.

Results: Average age: 38 years old, 58% women, 42% men. Asthma rating: Unrated 68%; Intermittent Asthma 12%, Moderate persistent asthma 2%, extrinsic or intrinsic asthma 16%. Spirometry: No spirometry 77%, with spirometry 23%. Only one patient had health education on inhalator uses. The most prescribed drugs: B2 short action (B2SA) 83%,

inhaled corticosteroids (IC) 75%, anti-leukotrienes (AL) 41%, B2 long action (B2LA) 27%. Round 45.8% were treated with two drugs, 27% with triple therapy, 16% as monotherapy and 8% with quadruple therapy. The most commonly used combination is the B2SA and IC in a 40%.

Conclusion: There are low use of spirometry for diagnosis and monitoring asthma patients, null health education on inhalators uses and finally nearly half of patients are being treated with dual therapy, being B2SA and IC the most frequently prescribed drugs.

P103-11

PROPERTIES OF FOUR HERBAL REMEDIES TRADITIONALLY USED TO TREAT ANXIETY

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Introduction: Traditionally, herbal remedies have been used to treat anxiety disorders. In addition, a renewed interest in herbal therapy has occurred over the last few decades and is having, consequently, an effect in scientific literature. Even though conventional usage and applications of herbal remedies are based on empirical evidence, personal experiences and tradition, scientific information to corroborate them needs to be provided.

Material and Methods: Going through scientific literature on the EM-BASE and PUBMED databases to determine whether or not traditional applications of the Aurantii flos (*Citrus aurantium*), Melissa folium (*Melissa officinalis*), Rhodanos flos (*Papaver rhoeas*) and Tiliae flos (*Tilia cordata* y *Tilia platyphyllos*) drugs, coincide with scientifically supported properties and instructions approved by the European Scientific Cooperative On Phytotherapy (ESCOP) and the German Commission E.

Results: No scientific literature validates the anti anxiety properties of the Rhodanos flos drug. The German Commission E does not consider the Tiliae flos drug to be an anti anxiety drug and ESCOP doesn't make references to it; however, in the scientific literature environment, ESCOP quotes *Tilia americana* var. *mexicana* as an anti anxiety drug. Aurantii flos has been used in animal testing and for aromatherapy as well. Melissa folium is cited together with other medicinal plants whose scientific properties have already been proved and it is also quoted in a children clinical test.

Conclusion: Except for the Melissa folium drug, scientific evidence to ratify the traditional usage of the studied herbal remedies is either very limited or inexistent. Nevertheless, such practices continue based mainly on the widely held belief that, because of their natural sources, herbs are innocuous.

P104-11

DETECTION AND ANALYSIS OF ADVERSE DRUGS REACTIONS IN HOSPITALIZED PATIENTS THROUGH THE MINIMUM BASIC DATA SET (MBDS)

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Introduction: Patient safety has become a public health priority, the main requirements of programs to improve patient safety include the capacity and quality to capture the fullest possible information on adverse drug reactions (ADRs) so that it can be used as tool for future preventive action. The aim of this work has been to study the number of ADRs occurred in hospitalized patients according to age and sex and to find out the mainly involved drugs on ADRs in women and men.

Material and Methods: Data were obtained from the Minimum Basic Data Set (MBDS) from patients treated and discharged between October and December 2010 in all health care services of the Hospital General Universitario Reina Sofía de Murcia (HGURS). We selected all hospitalization that was coded as complications and ADRs (ICD-9-CM codes E930-E949.9 MBDS). We also included those who had the code 995.2. As an indicator of severity of ADRs was selected average length of stay in the hospital of each episode.

Results: We analyze the drugs most frequently associated with ADRs, finding out that they were Acenocoumarol, Digoxin and Methylprednisolone. The study revealed that the occurrence of ADRs measured as the 'increase in the average hospital stay' differs by gender.

Conclusions: The MBDS has a great method for obtaining ADRs profile in a hospital but this requires the fullest possible involvement of professionals through the notification of the ADRs in the discharge report. The present findings demonstrated that the need for gender mainstreaming in health.

P105-11

HERBAL REMEDIES TRADITIONALLY USED IN THE COMUNIDAD VALENCIANA AS EXPECTORANTS AND ANTI-TUSSIVES. SCIENTIFIC EVIDENCE

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Introduction: In the Comunidad Valenciana, medicinal plants as red poppy, ratstail plantain, ivy and common horehound are used to treat and prevent respiratory disorders with cough and expectoration. The widely held belief that, because of their natural sources, herbs can't be harmful maintains or even increases their usage over the years.

Material and Methods: We have executed bibliographic searches in Pubmed and Embase to determine whether or not, in the Comunidad Valenciana, the traditional expectorant and anti-tussive properties of *Papaver rhoeas* L, *Marrubium vulgare* L, *Plantago lanceolata* L and *Hedera helix* L are supported by clinical tests and validated by the European Scientific Cooperative On Phytotherapy (ESCOP) and the German Commission.

Results: In the Comunidad Valenciana, *Papaver rhoeas* L and *Marrubium vulgare* L are used to treat respiratory disorders with cough and expectoration as the predominant symptoms. But neither of the herbal drugs is scientifically supported nor its properties are recognized by the ESCOP or the German Commission E. Nevertheless, *Plantago lanceolata* L and *Hedera helix* L are scientifically confirmed and ratified by both organizations.

Conclusion: The above herbal remedies have been used from generation to generation to treat respiratory complaints and colds. However, the traditional usage is not always endorsed by scientific research nor admitted by the Fitotherapy associations.

P106-11

CONSUMPTION OF DRUGS BY 65-YEAR OLD INDIVIDUALS IN THE REGION OF EL BIERZO

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Introduction: The use of drugs in the elderly has increased over the last years. The objective of the study was to evaluate the drug consumption and the extent of polypharmacy among 65-year old people living in the region of El Bierzo (León, Spain).

Material and Methods: Information on the intake of drugs was collected from 304 individuals randomly selected. The subjects were interviewed at the general practitioner's office.

Results and Conclusions: 93.6% of the population interviewed use some kind of drugs. The most common prescribed drugs were analgesics (70%), antihypertensive drugs (58%), hypocholesterolemic agents (51%) and hypnotics (42%). Of the over-the-counter drugs, 15% consumed medicines against gastrointestinal disorders and 7% analgesics. The average number of drugs in use was 3. Alternative medicine products (homeopathy, naturist medicine...) were also consumed by 15% of the participants. A high proportion of the study population consumed drugs. The observed polypharmacy may increase adverse events and medicinal interactions with clinical relevance in this group of population.

P107-11

USE OF ANTIPLATELET DRUGS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Controversy currently exists for the use of antiplatelet drugs in primary prevention in patients with type 2 diabetes.

Aim: To evaluate the prevalence to antiplatelet therapy in patients with type 2 diabetes.

Materials and Methods: Cross sectional study conducted in an urban health district level. The study subjects are patients with type 2 diabetes over the age of 30 who were not with anticoagulant treatment. The sample size (alpha risk of 0.05 to an accuracy of $\pm 0.035\%$ units in a two-sided for an estimated proportion of 0.25 and a replacement rate of 0.3) is 1100 patients. Collect data related to demographic and anthropometric, metabolic control (HbA1c), risk factors, cardiovascular events and drug therapy (focusing on antiplatelet therapy). Univariate analysis was performed with measures of central tendency and dispersion (quantitative variables) and frequency and percentage (qualitative variables) and bivariate analysis using unpaired *t* student and chi square test.

Results: The studied population had a mean age of 67, predominantly male (52%). Metabolic control was poor (31% of patients had HbA1c <7; 31% between 7 and 7.9; 38% ≥ 8). Patients had the following cardiovascular risk factors: hypertension (62%), dyslipidemia (42%; 85% with LDL levels above 100 mg/dl), obesity (39%) and smoking (10%). 80% were older than 80 years. A 62% of the patients were under antiaggregation (64% in primary prevention and secondary prevention 93%).

Conclusion: In the population of patients with type 2 diabetes studied the prevalence of antiplatelet use is low despite risk factors coexist with poor therapeutic control.

P108-11

OXIDATIVE STRESS IN PATIENTS UNDERGOING LIVER TRANSPLANTATION

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Currently provides a significant effect occurred etiopathogenic oxidative damage during hepatic ischemia-reperfusion in relation to primary graft dysfunction.

Objectives: To evaluate changes in oxidative status during liver transplantation.

Subjects and Methods: Prospective quasi-experimental study with a total of 39 patients. We included subjects who met criteria for liver transplantation, and excluded those with code indication O. Wisconsin was administered and Celsior solution in a non-random 20 and 19 patients respectively. The protocol of immunosuppression consisted of cyclosporine or tacrolimus with steroids. Biochemical parameters of oxidative stress: lipid peroxidation (TBARS), enzymatic activities (glutathione peroxidase, transferase and reductase) and glutathione levels (oxidized – GSSG-, reduced –GSH- and total glutathione –TG-) was determined.

Results: During reperfusion significantly increases the level of plasma TBARS (21.2 vs. 17.8 $\mu\text{mol/g}$ protein). GSH and TG in plasma decreased significantly after reperfusion (4.8 vs. 6.3 and 6.5 vs. 6.9 $\mu\text{mol/mg}$ protein, respectively). In liver tissue also find that the GT and GSH decreased significantly after reperfusion (42.4 vs. 46 and 58 vs 68 $\mu\text{mol/g}$ tissue, respectively). As for the liver enzyme activity were not found significant differences.

Conclusion: The data show an increase in the oxidative status and activation of antioxidant defense (increased percentage of the total oxidized glutathione) observed a depletion of reduced glutathione levels both peripheral and tissue during reperfusion.

P109-11

ACTIVATION OF CASPASE 3 BY 7-HYDROXYCOUMARIN IN TWO CELL LINES OF HUMAN LUNG ADENOCARCINOMA.

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Introduction: 7-hydroxycoumarin is the main biotransformation product of coumarin in humans. Coumarin and 7-hydroxycoumarin, at con-

centrations below 150 mg/ml, have an anti-proliferative effect in cells of human lung carcinoma, mediated by a decreased expression of cyclin D1 (Jimenez-Orozco FA et al, Lung Cancer 2001; 34:185-194). At concentrations over 150 mg/ml, 7-hydroxycoumarin produces morphological changes characteristic of apoptosis only on adenocarcinomas (López-González JS et al, Lung Cancer 2004; 43:275-283). The aim of this study was to identify the action mechanism by which coumarin and 7-hydroxycoumarin favour apoptosis in lung adenocarcinoma cell lines A-549 and A-427.

Materials: Coumarin and 7-hydroxycoumarin, human adenocarcinoma lung cell lines A-549 and A-427 (ATCC).

Results: Coumarin and 7- hydroxycoumarin treatment (2 mM, 24h) induces morphological changes characteristic of apoptosis in A-549 and A-427 cells. Treatment with 7-hydroxycoumarin induces an increase of 40% of caspase 3 enzymatic activity in A-549 cells and 12% in A-427 cells. Treatment with 7-hydroxycoumarin also induces an increase in cleavage of PARP and in the expression of Bax with a concomitant decrease of Bcl-2.

Conclusion: 7- hydroxycoumarin induces cell apoptosis, associated with the regulation of the Bcl-2 family proteins, enzymatic activation of caspase 3 and cleavage of PARP in both lung adenocarcinoma cell lines. These results indicate that coumarin and 7- hydroxycoumarin might be a prospect drug for the treatment of lung adenocarcinoma. Financial support granted by CONACYT (Mexico) 98729.

TEACHING IN PHARMACOLOGY

P110-12

FACEBOOK AS AN EDUCATIONAL TOOL IN PHARMACOGNOSY

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Facebook is one of the most popular social networking that can be applied as an innovative educational methodology to improve communication between teachers and learners and enhance learning competencies. The aim of the present study was to explore the use of Facebook as an online communication tool and its effectiveness to provide learning outcomes in Pharmacognosy, a compulsory subject in the third year of the Degree of Pharmacy of the University of Valencia. The study was conducted under a group of 45 students, three quarters of the registered ones. Once the Facebook group was created, different questions and problems on natural drugs, mainly on isolation and characterization of active plant secondary metabolites as well as their pharmacological properties, were formulated in the virtual classroom section. Members of the group try to solve them, what results in sharing knowledge. The methodology provided insight into the students' reflections as they emerged in the questions delivered to them. The teacher regularly responded, and the immediate messages imply checking the social network very frequently, so giving feedback to students. This tool was also applied to dispel doubts about the exam; to give detailed corrections and the marks obtained, therefore it extended the classroom to the virtual interface. A survey evaluated the students' experiences that joined the group. Overall, the community gained a positive experience from using Facebook. Provided that it is a persuasive strategy to improve professor-student and student-student relationships, it could work effectively as a complementary learning tool.

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P111-12

MONITORING STUDENT'S WORK IN A BLOG OF GENERAL PHARMACOLOGY

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In the last academic year, teachers developed a blog for the course "General Pharmacology", Faculty of Pharmacy, Barcelona (<http://www.farmacologiageneral2011.blogspot.com>), to guide the teacher-student relationship. Structure of the blog: It was divided into six sections: Home, Tips, Trivia, Hot Topic, Regarding today's class and autonomous work. To avoid unwanted intrusions, the blog was designed in such a way that it could not be found with a simple search on Google. To avoid being identified, students were advised to edit their own profile. Development of the blog: The blog was designed originally to monitor doubts and the work to be undertaken independently by the students. The section ¿questions about the class? received few comments, in spite of the fact that teachers continued to receive e-mails with doubts and office visits for consultations. The key section of the blog was autonomous work. 34.5% of students who performed the work were actively involved in this section. Initially the comments were about request for help, but gradually moved towards the sharing of problems and possible solutions.

Conclusions: In the section of autonomous work, a job that affects the final grade mark, students were seeking help and collaborating to the dynamism of the blog. Monitoring the blog was increasing throughout the semester. Identity preservation is not related to doubt exposure. By comparing the blog with the forum of the subject in the virtual campus, it follows that the direct teacher-student relationship is not a limiting factor, while it is the public exposure of the doubt ahead of their peers, even when it anonymously.

P112-12

THE COXIB'S TALE

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Introduction: We proposed a practical activity addressed to students of Pharmacotherapy in the 5th year of Pharmacy Degree with the main objective of favouring their critical reading of scientific information. The activity was designed to acquire the knowledge of the data management, therapeutic value, validation... as well as bioethical aspects of clinical trials.

Methods: The activity is developed in two sessions, in groups of 32 students working in computer classrooms with Internet access. The teacher proposed the reading of the following assays: CLASS (JAMA 2000;284 (10):1247-1255), VIGOR (N Eng J Med 2000; 343:1520-1528) and APPROVE (N Eng J Med 2005;352:1092-1102), which were accessible through the virtual platform of the University of Valencia. During the first face-to-face sessions, the students, working in subgroups (8), ought to look for additional information to answer different questions regarding the following aspects: financial disclosures, design, results, statistical analysis, conclusions and social impact of each study. At the final sessions, different groups discussed their findings and opinions answering the following questions: (i) 'What impact had the marketing and removal of rofecoxib in the media?'; (ii) 'What impact had the use of rofecoxib in Spain?' and (iii) 'Do Coxibs represent an advantage over traditional NSAIDs in the treatment of osteoarthritis?'

Results and Conclusion: An elevated proportion of the students have evaluated the sessions as a very positive activity to improve pharmacological knowledge and its practical usefulness.

P113-12

GOOGLEDOCS: AN ASSESSMENT INSTRUMENT OF COOPERATIVE WORK

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Introduction: Two problems encountered during the teaching of the master subject 'Advances in Pharmacology and Therapeutics' were the difficulties for the student's meetings and the inability of the teachers to objectively evaluate the participation of individual members of cooperative working groups. This has led us to consider the possibility of using Google Docs tool for solving both problems.

Objectives: To implement the use of Google Docs to promote the cooperative work of students in virtual form, and as a tool for continuous evaluation of the cooperative work.

Methodology: Students were asked to do a compulsory cooperative work (groups of four students) using the Google Docs platform to prepare a written review on a selected topic, and to present it orally.

Results: 60% of the groups shared their work with teachers, allowing them to see the working dynamics of the group. In contrast, some groups did not allow teachers to access the document until the work was already structured in a more advanced stage; each component of the group did his/her part in Word and uploaded it to the Google Docs platform. Students were also reluctant to display comments or notes that would show the real interaction of the group. The evaluation of the first groups was much more comprehensive, and teachers could interact effectively to redirect issues not properly addressed.

Conclusions: Google Docs perfectly fulfills the goals we had set, as a tool to assess the participation of each member of the group and it also facilitates collaborative work, but to be really effective students must be involved in their use.

P114-12
DEVELOPMENT OF A GUIDE OF PRACTICAL CASES ON PHARMACO-THERAPY MADE BY STUDENTS

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Problem based learning (PBL) is a teaching methodology in which the students use triggers of the case or problem to define their own learning objectives. It is a learning method related with the directives of the European Higher Education Area (EHEA) that we have already experienced in the teaching of practical lessons of Pharmacology and Pharmacotherapy. The proposed activity in the present project adds one more step to PBL methodology, since involves the design of the case by the students themselves. The students organized in groups, made a case or situation based on several keywords specified by the teacher, who oversees this work through various tutorial sessions, conducted in the classroom and warned in advance. Finally, the activity was completed with the selection of the best cases evaluated by the teachers and the subsequent development of a 'Guide of practical cases on Pharmacotherapy' designed by the students. The student participation in the planning case and its resolution is a way to involve them in their own learning. This forces them to try to assess which are the learning objectives of the subject and work it from a different perspective than usual. The degree of students satisfaction was assessed through surveys. The students highly appreciated the increase of their knowledge after having developed this activity.

References:

1. Bigelow J. J *Manag Educ* 2004;28:591–610.
2. Branda LA. El aprendizaje basado en problemas en la formación en Ciencias de la Salud. En: *El aprendizaje basado en problemas: una herramienta para toda la vida*. Agencia Laín Entralgo, Madrid; 2004, 17–2.

P115-12
ASSESSING MEDICAL STUDENTS' REFLECTION CAPACITY AND APPLIED KNOWLEDGE OF PHARMACOLOGY.

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It is expected that the knowledge of pharmacology acquired by medical students during the preclinical course will support the necessary reflection process they must perform during the practical clinical years in search for better therapeutic decisions. The objective of this study was to assess the extent in which medical students acquire the ability to use the basic knowledge through a questionnaire that induced a reflection process in the student (Viniestra L. *Rev Invest Clin* 1979; 31:413–420). A questionnaire of 79 questions on pharmacology of the so called false-true-do not know type, was applied to four groups of second year students (2202, 2205, 2227, 2229) and to a group of students in clinical internship as well. The questionnaire was also applied to two groups of students picked from the arts and humanities area. Groups 2202, 2205, 2227, 2229 and the group of clinical internship obtained an average score of 28, 44.5, 28.5, 28.0 and 43, respectively. On the other hand, the score obtained by the humanities students was 13.05±2.0, being significantly lower than the one obtained by medical students. It is concluded that the questionnaire distinguished medical students from students belonging to different academic areas. But also, the results suggest that some second-year students achieve an ability to apply knowledge on pharmacology comparable to that of the students in the clinical internship (PAPIME PE20409).