

ORAL ABSTRACTS

CARDIOVASCULAR PHARMACOLOGY

O01-01

CHANGES IN THE SEROTONINERGIC MODULATION OF THE PARASYMPATHETIC INNERVATION OF FOUR AND EIGHT-WEEK DIABETIC RAT HEART

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We have shown serotonergic enhancing actions (via 5-HT₃ receptor activation) and serotonergic inhibitory effects (via 5-HT₂ activation) of the bradycardia induced by vagal stimulation in normoglycaemic pithed rats. In the present work, we studied the changes induced in the serotonergic modulation of the parasympathetic innervation of the heart by 4 and 8-week diabetes. Diabetes was induced in male Wistar rats by a single s.c. injection of alloxan. Four and eight weeks later, animals were anaesthetized and pretreated with d-tubocurarine, atenolol, and pithed. Bolus i.v. administration of 5-hydroxytryptamine (5-HT) potentiated the bradycardia induced by electrical stimulation in 4-week diabetic rats; however, in 8-week diabetic rats, 5-HT had a dual effect on the bradycardia (potentiation at low doses and inhibition at high doses). 5-carboxamidotryptamine maleate (5-CT), 5-HT_{1/7} agonist, induced a dual effect both in 4- and 8-week diabetic rats. Enhancement of the bradycardia at 5-CT lower doses was reproduced by the 5-HT_{1A} agonist, 8-OH-DPAT, and abolished by WAY-100,635, 5-HT_{1A} receptor antagonist in both experimental models. The 5-CT inhibitory action was blocked by BRL15572, a 5-HT_{1D} antagonist, in 4-week diabetic rats; whereas, in 8-week diabetic rats, only pimoizide, a 5-HT₇ antagonist, was able to abolish 5-CT inhibitory action. In conclusion, short- and long-term diabetes elicited changes in the 5-HT receptor subtype involved in the modulation of the vagally-induced bradycardia. Activation of the 5-HT_{1A} receptors induces enhancement both in 4 and 8-week diabetic rats, whereas attenuation of the response is due to 5-HT_{1D} and 5-HT₇ receptor activation in 4 and 8 week-diabetic rats, respectively.

O02-01

ANAKINRA, AN INHIBITOR OF THE INTERLEUKIN-1 RECEPTORS, RESTORES THE ENDOTHELIAL FUNCTION ALTERED BY THE EXPERIMENTAL TYPE I DIABETES MELLITUS

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Introduction: We analyzed the role of pro-inflammatory cytokines, like interleukin-(IL)-1 β , in diabetic endothelial dysfunction, as well as the involvement of the NADPH-oxidase enzymatic activity.

Methods: Endothelium-dependent relaxations to acetylcholine (ACh; 1 nmol/l to 10 μ mol/l) were measured in isolated mesenteric microvessels of 2 weeks evolution streptozotocin-induced diabetic rats (60 mg/kg). Some animals were treated with the IL-1 blocker anakinra (AK; 100 mg/kg or 160 mg/kg per 24 h during 3 days). In some cases, before the curve to ACh, the vessels were pre-incubated during 30 min with 100 μ mol/l of tempol or 10 μ mol/l of apocynin.

Results: The impaired relaxations to ACh ($P < 0.003$) were partially reverted ($P < 0.01$) by treating the animals for 3 days with 100 mg/kg of AK. When the diabetic animals received 160 mg/kg of AK, the observed endothelial relaxation was restored, being similar to that observed in vessels from non-diabetic animals ($P < 0.001$). The independent-endothelial relaxations induced by sodium nitroprusiate (1 nmol/l to 10 nmol/l) were not altered in the diabetic animals with or without treatment with AK. The microvessels of diabetic rats pre-incubated with tempol or

apocynin showed a significant reduction of the endothelial dysfunction ($P < 0.001$), suggesting the participation of these mechanisms, which can be activated by a pro-inflammatory environment.

Conclusions: Experimental diabetic endothelial dysfunction in rats is mediated by pro-inflammatory cytokines, like IL-1 β since the endothelial function can be completely restored with the inhibitor IL-1-receptors anakinra. Furthermore, the mechanisms underlying these effects include the activation of the vascular NADPH-oxidase and the increase of superoxide anions.

O03-01

THE ANGIOTENSIN-(1-7)/MAS AXIS EXHIBITS ANTI-INFLAMMATORY PROPERTIES IN VASCULAR HUMAN SMOOTH MUSCLE CELLS

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Introduction: Angiotensin (Ang)-(1-7), considered as a physiological antagonist of Ang II, binds the G-coupled receptor Mas. We aimed to: (i) Establish the possible anti-inflammatory action of Ang-(1-7) in human vascular smooth muscle cells (HASMC) stimulated with Ang II or interleukin (IL)-1 β , as a renin-angiotensin system (SRA) independent-inflammatory molecule, (ii) Define the role of Mas receptor and identify signaling pathways involved in the anti-inflammatory action of Ang-(1-7).

Methods: Inducible nitric oxide synthase (iNOS) and nitric oxide (NO) levels were determined by Western blotting and Griess method, respectively. NADPH oxidase and nuclear factor (NF)- κ B activities were determined by luminescence and electrophoretic mobility shift assay, respectively.

Results: Both Ang II (100 nM) and IL-1 β (2.5 ng/ml) increased iNOS levels and NO release after 18 h. Also, both compounds triggered NADPH oxidase and NF- κ B activation. To establish the sequence of pro-inflammatory signaling by Ang II or IL-1 β , apocynin (30 μ M) and PDTC (100 μ M) were used. Both apocynin and PDTC reduced the levels of iNOS and NO release, while apocynin inhibited NF- κ B activation, therefore unveiling the sequential activation of NADPH oxidase, NF- κ B and iNOS by both stimuli. Pre-incubation with Ang-(1-7) (100 nM) markedly reduced both NADPH oxidase and NF- κ B activation, as well as iNOS induction and NO release by Ang II or IL-1 β . Furthermore, the Mas receptor antagonist A779 (1 μ M) totally blocked the anti-inflammatory effects of Ang-(1-7).

Conclusions: Ang-(1-7) via the Mas receptor attenuates inflammation of HASMC induced not only by Ang II, but also by other inflammatory molecule independent of the SRA.

O04-01

CARDIAC SODIUM CURRENT INHIBITION BY CARBON MONOXIDE

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Carbon monoxide (CO) has been suggested to cause fatal arrhythmias commonly characterized by elongation of the QT interval and early after-depolarizations, reminiscent of long QT syndrome. In the present study we have used conventional whole-cell patch clamp recordings to investigate the effects of CO on the recombinant human cardiac Nav1.5 channel stably expressed in HEK293 cells. CO inhibits the peak sodium current (I_{peak,Na}) without affecting the late sodium current (I_{L,Na}), despite the fact that I_{L,Na} can be increased in HEK293Nav1.5 cells by anemone toxin rATXII (40 nM) or 5-NO-2-pyridyldisulfide (DNTP, 50 μ M). CO inhibits the I_{peak,Na} in a concentration-dependent and voltage-independent manner (IC₅₀ = 1.05 μ M). Exposure to the CO donor CORM-2 (3 μ M) elicited a 71.3 \pm 4.7% decrease of basal I_{peak,Na} (mean \pm SEM, n = 21

cells), whilst 3 μM iCORM (the inactive form of the donor) had no significant effect. CO-mediated inhibition was dramatically reduced by the nitric oxide synthase (NOS) inhibitor L-NAME (1 mM, 1 h incubation) ($14.3 \pm 9.9\%$ inhibition, $n = 8$, $P < 0.01$ compare to % inhibition induced by 3 μM CORM-2 alone). CO-mediated inhibition was reduced by the reducing agents dithiothreitol (1 mM; $43.7 \pm 7.3\%$ inhibition, $n = 11$, $P < 0.01$) or L-cysteine (100 μM ; $48.6 \pm 9.0\%$ inhibition, $n = 7$, $P < 0.05$). Combination of L-NAME with reducing agents further blocked the CO-mediated effects on Ipeak,Na. Our results indicate that the inhibition of Ipeak,Na by CO is largely mediated by a rise of NO produced by activation of NOS, and suggest that the channel redox status (via cysteine groups) might be an important determining factor in this effect of CO. Financially supported by the British Heart Foundation.

O05-01 ANGIOTENSIN-II INDUCED-FRACTALKINE EXPRESSION AND MONONUCLEAR CELL RECRUITMENT IN HUMAN ARTERIAL ENDOTHELIAL CELLS IS TNFA-MEDIATED

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Background: Fractalkine/CX₃CL1 (FR) is a membrane-bound chemokine and an adhesion molecule implicated in vascular inflammation. In previous studies we demonstrated that angiotensin-II (AII) increases FR expression and mononuclear cell recruitment. Objective: To investigate the possibility that endogenous endothelial TNF α influences the AII-responses.

Methods: Human umbilical arterial endothelial cells (HUAECs) were stimulated with AII (1 μM , 24 h). In some experiments, cells were transfected with a specific siRNA to knock-down TNF α expression or with its respective control. Flow cytometry was used to analyze FR expression and flow chamber to evaluate the functional role of TNF α on AII-induced mononuclear cell arrest under dynamic conditions.

Results: Fourty eight hours posttransfection with TNF α -specific siRNA, HUAECs showed a > 80% reduction in TNF α mRNA expression, compared with the control siRNA-transfected cells. Similarly, TNF α siRNA resulted in a > 70% decrease in intracellular TNF α protein relative to control-treated cells, indicating that TNF α siRNA transfection successfully suppressed most of TNF α expression in HUAEC. AII stimulation significantly increased the expression of FR and caused mononuclear cell recruitment in control siRNA-transfected cells, but not in TNF α -deficient HUAEC.

Conclusions: These results suggest that TNF α is involved in AII-induced FR expression on arterial endothelial cells and the subsequent mononuclear cell recruitment. The design of new drugs targeting FR or TNF α function might reduce the vascular inflammation associated with rennin-angiotensin system activation. This work was supported by grants SAF2008-03477, SAF2009-08913 and PI08/1875, RIER RD08/0075/0016, from Spanish Ministry of Science and Innovation, Carlos III Health Institute, Spanish Ministry of Health and other grants from Generalitat Valenciana.

O06-01 MECHANISM OF METOPROLOL-MEDIATED SELECTIVE INHIBITION OF ADENYLYL CYCLASE 5 (AC5) IN HL-1 CARDIOMYOCYTES. ROLE OF ALPHA1A ADRENERGIC RECEPTORS (ALPHA-1A-AR) AND CALCIUM CALMODULIN KINASE II (CAMKII)

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Introduction: Previous data of our lab showed a selective inhibition of AC5 by norepinephrine in the presence of metoprolol in HL-1 cardio-

myocytes. This finding is relevant considering the deleterious effects of this isozyme in heart failure pathophysiology. The underlying mechanism of this selective inhibition is unknown. The goal of the present study was to assess whether alpha1 and beta1 adrenergic interaction was related to this effect.

Material and Methods: HL-1 cardiomyocytes were preincubated with norepinephrine (10⁻⁷ M) in the presence of metoprolol (10⁻⁵ M) dissolved in Krebs Henseleit + IBMX (0.5 mM) for 10 min. The antagonists and inhibitors were added 10 min before and were present during the preincubation. After three washes, cardiomyocytes were exposed to isoproterenol (10⁻⁶ M) for another 10 min. Finally, cAMP accumulation measures were carried out in triplicate.

Results: The inhibition on isoproterenol-induced cAMP accumulation mediated by norepinephrine and metoprolol was reverted by the alpha1A-AR selective antagonist WB 4101 (10⁻⁷ M) ($P < 0.05$) but not by the alpha1B- and alpha1D-AR antagonists chloroethylclonidine (10⁻⁵ M) and BMY 7378 (10⁻⁸ M). The pertussis toxin pretreatment (0.5 $\mu\text{g/ml}$) did not antagonize the inhibition induced by norepinephrine and metoprolol. alpha1A-AR-mediated inhibition was reverted by CaMKII inhibitors (AIP; 2×10^{-5} M and KN-62; 10⁻⁵ M; $P < 0.05$) but not by PKA (H89; 3×10^{-6} M) or PKC (GF 109,203X; 10⁻⁶ M) inhibitors. AIP blocked the inhibition when forskolin was used to stimulate AC activity ($P < 0.05$).

Conclusion: alpha1A-AR-CaMKII pathway is involved in norepinephrine-mediated inhibition of AC5 in the presence of metoprolol.

O07-01 LOW CONCENTRATIONS OF TRANS-RESVERATROL INDUCE THE RELEASE OF CALCIUM FROM THE ENDOPLASMIC RETICULUM THROUGH A NITRIC OXIDE/CGMP-DEPENDENT PATHWAY IN

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Inhibition of adrenal catecholamine release by resveratrol has also been implicated in its cardioprotecting effect. In a recent publication, we have suggested that this effect could be mediated by an intracellular target beyond the last Ca²⁺-dependent exocytotic steps. We have also observed that resveratrol causes elevation of [Ca²⁺]_c, and this effect could be linked to cGMP activation. With all these previous observations, the aim of this study was to investigate the intracellular mechanism of resveratrol (1 μM) by which it produces [Ca²⁺]_c increase in BCCs. We have found that: (i) Pretreatment with ryanodine, thapsigargin and caffeine abolished the calcium transient elicited by resveratrol; (ii) This effect was also blocked by ryanodine or dantrolene; (iii) ODQ and L-NAME produced a reversible antagonism of the resveratrol response; (iv) Measurement of nitrites demonstrated that resveratrol induced a concentration-dependent nitrite formation, which was also blocked by L-NAME. In addition, SNAP was also able to evoke a [Ca²⁺]_c transient; (v) Finally, resveratrol induced NO production which was also inhibited by L-NAME. Therefore, our results suggest that resveratrol activates NOS and consequently NO production in BCCs. On the one hand, NO has a well-established functional inhibitory role on catecholamine secretion evoked by acetylcholine or high K⁺. On the other, NO-release induced by resveratrol can increase [Ca²⁺]_c by release from internal ryanodine-dependent stores. In conclusion, we have demonstrated that resveratrol activates NO/cGMP-dependent pathway in BCCs that might be related to its ability to mitigate catecholamine release; this effect could contribute to the reputed cardiovascular protecting effect of resveratrol.

O08-01**INDUCTION OF G PROTEIN-COUPLED RECEPTOR KINASE-5 (GRK5) MEDIATED BY CNN2/CTGF IN HUMAN HEART FAILURE AND ISOLATED STEM CELLS FROM MOUSE MYOCARDIAL TISSUE**

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Background: Myocardial connective tissue growth factor (CNN2/CTGF) is repressed in healthy hearts, while its expression is induced in heart failure. Previous studies have demonstrated that CTGF may increase cardiac myocyte expression and activities of G-protein-coupled receptor kinase-5 (GRK5), an important regulator of β -AR responsiveness, and exert cardioprotective actions (Ahmed, MS, et al. *Am J Physiol Heart Circ Physiol* 2011).

Aims: (i) To determine the expression of GRK5 in isolated mouse myocardial stem cells treated in the absence or presence of recombinant human CTGF; (ii) To investigate myocardial expression of CTGF and GRK5 in samples from left ventricle of human failing hearts and biopsies from transplanted healthy hearts and assess correlation with clinical parameters of cardiac function, as well as the putative correlation between CTGF and GRK5.

Methods: Stem cells were stimulated with rhCTGF and RNA was isolated using Qiagen columns. RNA was isolated from left ventricle (n = 39) and biopsies (n = 20) using Tripure Isolation Reagent. mRNAs encoding the GRK5 and CTGF genes was quantified by TaqMan[®] real-time PCR. Furthermore, protein isolation from stem cells treated with rhCTGF was extracted to Western Blot studies.

Results: There is a significant increase in myocardial expression of CTGF in failing human hearts (P < 0.001) which correlates with GRK5 levels and inversely with parameters of cardiac function. The data is consistent with the hypothesis that CTGF stimulates increase of GRK5 expression in cardiac stem cells/progenitors and contributes to cytoprotection.

Conclusions: This study explains a possible mechanism of control of critical β -adrenergic responsiveness through CTGF-mediated regulation of GRK5.

O09-01**PERIPHERAL BLOOD MONONUCLEAR CELLS FROM COPD PATIENTS SHOW INCREASED CX₃CR1 EXPRESSION AND BIND TO CIGARETTE SMOKE EXTRACT-STIMULATED HUMAN ARTERIAL ENDOTHELIAL CELLS**

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Background: Fractalkine/CX3CL1 (FR) is a relevant chemokine involved in leukocyte recruitment within the cardiovascular system.

Objective: To investigate the expression of FR receptor (CX3CR1) in circulating leukocytes from chronic obstructive pulmonary disease (COPD) patients and evaluate their adhesiveness to human umbilical arterial endothelial cells (HUAEC).

Methods: Flow cytometry was used to analyze CX3CR1 receptor expression, flow chamber to evaluate their functional role and FR circulating levels were determined by ELISA.

Results: Whole blood from healthy volunteers and COPD patients (FEV1/FVC < 70% and FEV1 < 30%) were obtained from 20–21 subjects in each group. Flow cytometry analysis revealed increased CX3CR1 expression on circulating monocytes and lymphocytes from COPD patients. However, FR circulating levels remained unchanged in both groups. When whole blood was perfused across HUAEC stimulated or not with 1% cigarette smoke extract (CSE) for 24 h, significant

increases in leukocyte adhesion were observed in stimulated HUAEC regardless the group investigated. Neutralization of FR activity resulted in a significant reduction of CSE-induced leukocyte adhesion being this effect more patent for leukocytes from COPD patients than for those from healthy individuals.

Conclusions: These results suggest that leukocytes from COPD patients express functional CX3CR1 receptor that may explain the increased leukocyte adhesiveness to arteries distant from the lung. Therefore, targeting FR/CX3CR1 axis might prevent cardiovascular diseases in COPD patients.

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O10-01**CYCLOOXYGENASE DECREASES NITRIC OXIDE AND INCREASES CONTRACTILE PROSTANOIDS IN RESPONSE TO THROMBOXANE A2 IN AORTA FROM AGED FEMALE MICE**

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Introduction: Throughout ageing release of nitric oxide (NO) is compromised in female. We used aortas from senescence-accelerated mouse (SAM), a standard model of ageing, to evaluate the contribution of prostanooids and NO in contractile responses to U46619, a stable analogue of thromboxane A2.

Methods: Six months old senescent-resistant (SAMR1, n = 7) and senescent-prone (SAMP8, n = 7) were used. Vascular rings from thoracic aorta were mounted for isometric recording of tension and concentration-response curves to U46619 (10⁻⁹–10⁻⁷ M) were performed in the absence and in the presence of NO synthase inhibitor NG-nitro-L-arginine methyl ester (L-NAME, 10⁻⁴ M) and/or unspecific cyclooxygenase inhibitor indomethacin (10⁻³ M). A segment of aorta from each mouse was frozen for immunofluorescence and protein expression analysis.

Results: U46619 evoked higher contractile responses in aortic segments from SAMP8 than SAMR1, that were increased after incubation with L-NAME in SAMR1 and slightly, but significantly, in SAMP8, suggesting a decreased bioavailability of NO in senescent mice. Indomethacin decreased contractions to U46619, indicating an involvement of contractile prostanooids in the aortic response of both strains of female mice. After the co-incubation of segments with L-NAME and indomethacin response to U46619 reached control values.

Conclusions: Ageing decreased NO and increased contractile prostanooids in response to U46619 in female aorta. Cyclooxygenase inhibition increases NO bioavailability counteracting the deleterious effects of female vascular ageing. Supported by Ministerio de Ciencia e Innovación, ISCIII (FIS 10/00518, RED HERACLES RD06/0009); Conselleria de Sanidad, Generalitat Valenciana (AP-131/10, AP-117/10 and GE-021/10).

O11-01**CHANGES IN THE EXPRESSION LEVELS OF ENOS DURING CULTURE OF ISOLATED CELLS AND ANGIOGENESIS**

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Angiogenesis, the growth of new blood vessels, is regulated by macrophages, pericytes, and fibroblasts through a complex molecular cascade in aortic cultures. Angiogenic factors induce the release of nitric oxide

(NO), although the role of this mediator in angiogenesis still remains unclear.

Aims: To determine the eNOS expression in the different cell types present in a vessel and its change during culture and angiogenic growing.

Methods: Rings from the aorta of Wistar rats were seeded on polymerized Matrigel[®] and cultured in EGM-2MV medium (37°C, 5% CO₂) for 7 days. Endothelial and smooth muscle cells (SMC) cells from rat aorta and caudal artery or fibroblasts from heart, were isolated and cultured under different conditions (Kipshidz N. et al 2000; Grobmyer SR. et al 1993; Ju H. et al 1998). mRNA for eNOS was determined by qPCR (Oliver E. et al 2010).

Results: Expression of eNOS in fresh SMC from aorta was 220.92 ± 53.20, but disappeared after 5 days of culture. Similar results were observed for SMC from caudal artery, fibroblasts, or even in isolated EC cultured under different conditions. Nonetheless after a 7-day angiogenic growing of aorta, eNOS was expressed in EC.

Conclusion: Irrespectively of cell type and tissue, isolated SMC, EC or fibroblast led to a loss of eNOS expression in culture. However under the same conditions, eNOS remained expressed when the entire vessel was cultured during the angiogenic process, suggesting a role for NO in the communication between different cell types. D Vicente received a fellowship from Spanish Ministry of Education and Science

O12-01 MECHANISM OF A LESPEDEZA CAPITATA EXTRACT (MICIFRONA[®])-INDUCED VASORELAXATION IN THE RAT AORTA

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Introduction: The present study was aimed to investigate de vascular effects of a *Lespedeza capitata* extract (LCE; Micifrona[®]) on rat isolated aortic segments and the possible mechanisms involved in such effects. LCE, presently used in renal urenic diseases, contains a number of flavone derivatives (1%) being the main lescapicoside.

Materials and methods: Rat helical thoracic aortic segments (2 mm in length) were mounted in organ baths at 37°C containing Krebs-Hepes solution. Arterial segments were stretched to an optimal resting tension of 1 g. Isometric tension was recorded using a force displacement transducer connected to an acquisition system.

Results: LCE (0.025–12.5 µg/ml) caused a concentration-dependent relaxation of aortic strips precontracted with different contractile stimuli (KCl, noradrenaline, phenylephrine). This effect was significantly reduced by endothelium removal, and after pretreatment with N(G)-nitro-L-arginine methyl ester (L-NAME, 100 µM). LCE-elicited varorelaxation was dependent on the sGC/cGMP pathway as the treatment of aortic strips with an inhibitor of the soluble guanylyl cyclase (1H-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one; ODQ, 10 µM), abolished the vasorelaxation induced by this drug. The vasorelaxation on noradrenaline-induced contraction was non-competitive. These data show for the first time, that the vasodilation induced by Micifrona[®] occurs through the production of endothelium-derived NO, and the further activation of the production of cGMP by this signaling molecule. These vasodilator effects may be responsible for the beneficial effects that this drug has on the physiopathology of the renal system, such as its effect as a mild diuretic, antiuremic and antihiperazotemic, ability to dissolve kidney stones and, with antispasmodic effects.

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O13-01

INTRACORONARY AUTOLOGOUS PERIPHERAL STEM CELL THERAPY FOR PEDIATRIC DILATED CARDIOMYOPATHY: BRIDGE TO TRANSPLANTATION OR TO RECOVERY?

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Introduction: Nearly one-third of children diagnosed with dilated cardiomyopathy (DCM) receives a transplant or die within the first year after diagnosis. Limited long-term outcome of heart transplantation and scarcity of young donors justify every effort for improving medical therapy and finding alternative therapeutic approaches. We describe the compassionate use of intracoronary autologous peripheral stem cell therapy in two young infants diagnosed with DCM and severe heart failure.

Patients: Two pediatric patients (a 3-month male infant weighing 4 Kg. and a 4-month male infant weighing 5 Kg.) diagnosed with DCM and severe heart failure, treated with intracoronary infusion of autologous stem cells.

Results: Both patients were in poor clinical status (NYHA IV), had high levels of NT-ProBNP, severe LV dilation and systolic dysfunction (EF < 30%). Both were mechanically ventilated and needed IV inotropes. Endomyocardial biopsy ruled out myocarditis. After treatment with G-CSF for 4 days, autologous stem cells were recovered from peripheral blood, implanting them afterward via coronary arteries. One week after the procedure, echocardiography showed a remarkable improvement in LV dilation. One month later a significant gain in their EF (>40%) was detected and maintained over time. The long-term outcome was favorable. Although first patient has shown LV dilation 17 weeks after the procedure, he is still in functional class I and has adequate weight gain.

Conclusions: Although currently heart transplantation is the only established treatment for DCM, stem cell therapy could be an option to improve clinical status.

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O14-01

THE ROLE OF INDUCIBLE NITRIC OXIDE SYNTHASE IN THE LOSS OF THE CONTRACTILE RESPONSE INDUCED BY PHENYLEPHRINE IN ISOLATED RAT THORACIC AORTA

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Introduction: Endothelial modulation of agonist-induced vasoconstriction in vessels has been reported (1,2,3). The aim was to investigate the role of nitric oxide on the 'desensitization' of the contractile response to α 1-adrenoceptor (α 1-AR) stimulation in rat thoracic aorta.

Material and Methods: Functional studies in organ bath were done to determine the contraction response to phenylephrine (α 1-AR agonist): two consecutive concentration-response curves (CCR) were obtained under different conditions. We performed real-time RT-PCR to quantify the expression levels of inducible, endothelial, and neuronal NOS (iNOS, eNOS, and nNOS) after obtaining the first CCR to phenylephrine.

Results: The maximal contraction to phenylephrine was significantly reduced in the second CCR ($56.7 \pm 0.6\%$ KCl80 mM, vs. $91.2 \pm 5.6\%$ KCl80 mM $n = 5$, $P < 0.01$) after a 4–5 h incubation in the bath. This hyporeactivity was also observed when the phenylephrine-induced maximal contraction was relaxed by isoprenaline (β -agonist), or even when the first phenylephrine-CCR was not performed. Application of an NOS inhibitor (L-NAME 100 μ M) recovered the contractile response to the agonist, whereas L-NAME had no effect on the basal tone. The iNOS mRNA levels increased under the different experimental conditions if compared to the freshly isolated aortic rings, suggesting that organ bath incubation conditions enhance the iNOS expression. No significant change in the eNOS and nNOS expressions was detected.

Conclusion: NO, produced by increased iNOS expression may be responsible for the vascular hyporeactivity of rat aorta to phenylephrine over time.

References:

- (i) Fleming I. et al., Proc Acad Sci USA 1999; 96:1123–1128;
- (ii) Gürdal H. et al. Br J Pharmacol 2005; 45:203–210;
- (iii) Jin X. et al. J Pharmacol Sci 2008; 108:95–103.

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O15-01

DELETERIOUS EFFECTS OF OXIDIZED LDL ON DDAH/ADMA/NO PATHWAY IN HUMAN ENDOTHELIAL CELLS ARE REVERTED BY ESTRADIOL INVOLVING ESTROGEN RECEPTOR ALPHA

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Introduction: Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide (NO) synthase. ADMA accumulation, mainly due to a decreased dimethylarginine dimethylaminohydrolase (DDAH) activity, has been related to the development of cardiovascular diseases. Estradiol exerts cardiovascular protective effects via the promotion of endothelial vasodilator synthesis. We investigate whether estradiol prevents the alterations induced by oxidized low density lipoprotein (oxLDL) on the DDAH/ADMA/NO pathway in human umbilical artery endothelial cells (HUAEC).

Methods: HUAEC were exposed to estradiol (1 nM), native LDL (nLDL, 100 μ g/ml), oxLDL (100 μ g/ml) for 24 h. Role of estrogen receptors (ER) was studied through unspecific antagonist ICI182780 and specific ER alpha receptor (MPP). ADMA concentration was measured by high-performance liquid chromatography (HPLC) and concentration of NO by an amperometric method. Protein expressions and DDAH activity were measured by immunoblotting and an enzymatic method, respectively.

Results: Exposure of HUAEC to oxLDL, but not to nLDL, decreased DDAH activity with a concomitant increase of ADMA concentration. oxLDL reduced both eNOS protein and NO production. Estradiol alone

had no effects on DDAH/ADMA/NO pathway, but increased the attenuated endothelial NO production induced by oxLDL as a consequence of the restoration of DDAH activity. Estradiol effects were abolished in the presence of both ICI182780 and MPP.

Conclusions: Estradiol restores DDAH activity ADMA levels and NO production impaired by oxLDL in HUAEC through the activation of ER alpha. Supported by Ministerio de Ciencia e Innovación, ISCIII (FIS 10/00518, RED HERACLES RD06/0009/0005); Conselleria de Sanidad, Generalitat Valenciana (AP-117/10 and GE-021/10).

O16-01

OXIDATIVE STRESS IS INCREASING IN PATIENTS UNDER CORONARY ARTERY BYPASS GRAFTING SURGERY AND PREDICTS A HIGHER CORONARY RISK

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Introduction: Oxidation enzymatic mechanisms in humans can be physiologically altered due to inflammatory or infectious stimuli to the organism. We studied the variations of multiple oxidative stress related products and enzymes in a cohort of patients who underwent coronary artery bypass grafting surgery (CABG).

Materials: We measured the concentration of malondialdehydic acid (MDA), nitrates, peroxynitrites (PT), reduced glutathione (RG) and mitochondrial superoxide dismutase (SOD-Mn) 2 h before and 24 after CABG.

Results: One hundred and eighty patients were included in this study. Statistically significant differences were detected in the mean plasmatic MDA concentration before (0.148 mmol/l /SD 0.12) and after (0.283 mmol/l /SD 0.16) surgery ($P < 0.001$). Higher concentrations of PT ($P = 0.443$) and nitrates ($P = 0.078$) were also detected, differences did not reach statistical significance (DNSS). Lower levels of RG and SOD-Mn were detected after surgery ($P = 0.94$ and $P = 0.070$), NDSS Hematite MDA and nitrite levels were independent predictors of greater Framingham/ATP III scale punctuations ($B = 32.146$ points/mmol/ml (IC 95% 24.132–40.16); $B = 0.326$ points/ μ mol/g (IC 95% 0.049–0.604)). GSH levels were inversely related to Framingham/ATP III punctuation [$B = -0.175$ points/ μ mol/g (IC 95% = -0.344 to -0.005)]. No significant relationship was detected between SOD-Mn ($P = 0.559$) or peroxynitrites ($P = 0.721$) and Framingham/ATP III risk.

Conclusions: Coronary artery bypass grafting surgery worsens the oxidative stress in patients with coronary disease higher oxidation activity and greater concentrations of its products and a lower antioxidant activity. MDA and nitrite levels predict greater Framingham/ATP III scale punctuations. On the contrary, GSH levels are related to lower Framingham predicted coronary risk.

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PHARMACOLOGY OF PAIN AND INFLAMMATION

O17-02

SENSITIVITY TO ANTINOCICEPTION MEDIATED BY THE μ OPIOID RECEPTOR IS DETERMINED BY CROSS-REGULATION BETWEEN μ AND δ OPIOID RECEPTORS

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Introduction: The perception of pain and its inhibition varies considerably between individuals and this variability is still unexplained. The aim of the present paper is to determine whether functional interactions between opioid receptors (OR) are involved in the interindividual variability in the sensitivity to μ -OR agonists.

Material and Methods: Antinociceptive tests, radioligand binding, stimulation of [³⁵S]GTP-gamma-S binding, inhibition of cAMP production and co-immunoprecipitation experiments were performed in two strains of rat (Sprague Dawley bred at our University SDU and Wistar) that differ in their sensitivity to opioids.

Results: delta-OR determine the enhanced sensitivity to antinociception induced by μ -OR agonists in SDU rats, because the increased potency of μ -OR agonists in this strain is reversed by naltrindole. Differences are found between SDU and Wistar brain membranes in inhibition experiments of the binding of [³H] naltrindole by μ -OR agonists. Differences are also evident in the effect of δ -OR ligands on the binding of [³⁵S]GTP- γ -S stimulated by μ -OR agonists. The potency of morphine in the inhibition of cAMP production varies between these strains when it is measured in the presence of deltorphin II and naltrindole. Co-immunoprecipitation experiments demonstrate that δ -OR are associated in a higher extent to μ -OR in SDU than in Wistar synaptosomal fraction.

Conclusions: The results obtained demonstrate that the increased cross-talk between μ and δ -OR in SDU, as compared to Wistar rats, is related to an enhanced sensitivity to μ -OR agonists induced antinociception.

O18-02

ANTI-INFLAMMATORY AND BONE PROTECTIVE EFFECTS OF CORM-3 IN POSTMENOPAUSAL ARTHRITIS

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Introduction: CO-releasing molecules (CO-RMs) are a new class of drugs able to release small amounts of CO in biological systems. We have shown previously the anti-inflammatory effects of CORM-3 in animal models. The aim of this study was to assess the effects of CORM-3 on bone metabolism in a model of postmenopausal rheumatoid arthritis osteoporosis.

Methods: Ovariectomy was followed by collagen-induced arthritis in female DBA-1/J mice. CORM-3 (10 mg/kg/day) and ALN (100 μ g/kg/day) were administered from day 22. The clinical score was studied using a scale of 0–2 in each paw. Mice were killed at day 36 or 50. Serum levels of different mediators were measured by ELISA or luminex. Hind paws were homogenized for measurement of inflammatory mediators. Trabecular microarchitecture was analyzed by μ CT.

Results: The arthritic score was significantly reduced by CORM-3 but not by ALN treatment. Local bone erosion and reduction in TNF α levels were seen for CORM-3 on day 50 and ALN on day 36. Serum levels of COMP, IL-6, MMP-3, alkaline phosphatase and osteocalcin were

decreased by both treatments, whereas TNF α levels were reduced by CORM-3 and TRAP-5b by ALN. Micro-computed tomography analysis showed protective effects on trabecular bone which were more prominent for CORM-3 on day 36 and for ALN on day 50.

Conclusions: CORM-3 shows anti-inflammatory effects and is able to reduce systemic bone loss and joint erosion in an animal model of OVX+CIA. These data suggest that CO-RMs could be an effective strategy on postmenopausal arthritis.

O19-02

DEVELOPMENT OF A POSTMENOPAUSAL OSTEOARTHRITIS MODEL IN ADULT FEMALE WISTAR RATS

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Introduction: Osteoporosis and osteoarthritis are more common in postmenopausal women due to estrogen deficiency. Current treatments are not effective enough to treat both diseases. In this study, we developed a rat animal model in which we induced both diseases to expand current knowledge about the mechanisms involved and to develop new treatments.

Materials and Methods: Twenty-five 8 week-old female Wistar rats (150–170 g), were randomly assigned into three groups: BNO (control), OVX (ovariectomy) and OVX+ACLT (ovariectomy followed by anterior cruciate ligament transection, 14 days later). The animals were killed at 14 weeks and the paws were examined by μ -CT, or they were homogenized to measure inflammatory mediators and biomarkers by ELISA, RIA and Multiplex Luminex. IL-6, IL-17, IL-1 β , TNF α and PGE₂ were measured in homogenized paws and osteoprotegerin (OPG), RANKL, osteocalcin, Cartilage Oligomeric Matrix Protein (COMP), Tartrate-Resistant Acid Phosphatase 5b (TRAP5b), alkaline phosphatase and PGE₂ were measured in serum.

Results: The results showed an increase of weight in the OVX group. The proinflammatory cytokines IL1- β , IL-6 and IL-17 increased significantly in OVX+ACLT group whereas OPG/RANKL ratio and TRAP5b decreased significantly. Articular cartilage degradation and trabecular bone loss was observed in this group. The levels of the cartilage biomarker COMP were enhanced and no significant changes in the levels of other mediators were observed in OVX+ACLT group.

Conclusion: This model can be used to study the mechanisms involved in both diseases and to identify possible therapeutic targets and new treatments for osteoarthritis in postmenopausal women.

O20-02

DP1 RECEPTOR ACTIVATION AMELIORATES THE ARTHRITIC PROCESS IN CIA

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Introduction: Collagen-induced arthritis (CIA) mice is an animal model of rheumatoid arthritis widely used to test potential therapeutic agents. PGD2 plays a complex role in inflammation acting as an inflammatory mediator although in some circumstances it can exert anti-inflammatory effects. This prostanoid exerts its effects principally by binding and activating two D type prostanoid receptors: DP1 and DP2. The aim of this study is to investigate the role of DP1 and DP2 receptors and their modulation in the evolution of arthritis.

Methods: Collagen-induced arthritis was induced in DBA/1J mice and the evolution of the inflammatory response was studied from day 0 to 34 after the induction of the arthritis. DP1 (BW245C) or DP2 (13,14-dihydro-15-ketoPGD2) agonists were administered from days 21 to 33. On day 34, serum samples were obtained and animals were sacrificed. Hind paws were homogenized for measurement of inflammatory mediators or were used for histological analysis.

Results: Treatment of animals with the DP1 agonist decreased the incidence and the severity of arthritis whereas administration of the DP2 agonist increased the clinical score of CIA. Local levels of IL-1 β and CXCL-1 were significantly reduced by the DP1 agonist whereas IL-17 production was inhibited by both agents. In addition, BW245C showed a tendency to increase IL-10 production.

Conclusions: Our data indicate that DP1 is the main receptor involved in the regulatory effects of PGD2 in murine CIA, as the DP1 activation ameliorated the arthritic process and regulated the production of pro-inflammatory mediators and cell migration into the joint.

O21-02

HO-1 INDUCTION BY COPP DOWN-REGULATES THE INFLAMMATORY AND DEGENERATIVE OSTEOARTHRITIC PROCESS IN HUMAN OSTEOBLASTS

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Introduction: The osteoblasts participate in subchondral bone remodeling and bone mineralization during osteoarthritis (OA). Heme oxygenase-1 (HO-1) exerts protective effects in OA joint by decreasing the production of inflammatory and degenerative mediators. We have studied the implication of HO-1 in the regulation of osteoblasts during OA.

Patients and methods: Cells were isolated from the trabecular area of the tibial plateau (seven OA patients) and cultured in osteogenic medium. Osteoblasts (third passage) were treated with the HO-1 inducer cobalt protoporphyrin IX (CoPP, 10 μ M) in the presence or absence of IL-1 α (10 ng/ml) for 3 h (mRNA determination) or 4 and 24 h (other determinations). Matrix mineralization was analyzed by Alizarin red incorporation, gene expression by quantitative PCR, protein expression by Western Blot and ELISA, PGE2 by RIA and matrix metalloproteinase (MMP) activity by fluorometry.

Results: Analysis of protein expression and mRNA showed that IL-1 β down-regulated HO-1 expression with respect to basal conditions. These effects were significantly reverted by CoPP. HO-1 induction significantly reduced PGE2 production, COX-2 and mPGEs-1 induced by IL-1 β . IL-6, TNF- α , MMP activity and mRNA expression of MMP-1, 2 and 3 were significantly increased by IL-1 α and reduced by CoPP. Also, HO-1 induction significantly enhanced bone mineralization and mRNA expression of osteocalcin, osteopontin, osteoprotegerin and collagen IA1 and IA2, with respect to IL-1 β .

Conclusion: We have shown that HO-1 decreases the production of relevant inflammatory and catabolic mediators that participate in OA pathophysiology, suggesting that HO-1 is a pharmacological target to control the activity of osteoblasts.

O22-02

MU-OPIOID RECEPTORS AND NMDA RECEPTORS ASSOCIATE IN PAG AND SPINAL CORD NEURONES: IMPLICATIONS IN NEUROPATHIC PAIN CONTROL

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The capacity of opioids to alleviate inflammatory pain is negatively regulated by the glutamate-driven N-methyl-D-aspartate receptor (NMDAR). An increased activation of NMDARs is responsible for the neural changes accompanying nerve-injury-induced neuropathy, diabetic neuropathy, chronic inflammatory pain, cancer pain, and post herpetic pain. Unfortunately, in these conditions mu-opioid receptor (MOR)-activating opioids do not provide an efficacious pain relief. Ultrastructural studies have demonstrated the co-existence of both receptors within single neurones of the CNS, including those in the mesencephalic periaqueductal grey (PAG) and dorsal spinal cord, regions implicated in

the opioid control of nociception. We now report that MORs and NMDAR NR1 subunits associate in postsynaptic structures of these neurones. Morphine disrupts the MOR-NMDAR complex and potentiates the NMDAR-calcium and calmodulin dependent kinase II (CAMKII) pathway that is implicated in morphine tolerance. Inhibition of protein kinase C (PKC) restores the MOR-NR1 association and rescues the analgesic effect of morphine. Conversely, administration of N-methyl-D-aspartic acid segregates by a PKA-dependent mechanism the MOR-NR1 complex, it also uncouples the MOR from the regulated G proteins, and the antinociceptive capacity of morphine results greatly diminished. These molecular changes as well as the reduction of morphine analgesia are also observed in sciatic nerve-injured mice. Inhibition of PKA blocked these effects and preserved morphine antinociception. Thus, the opposing activities of the MOR and NMDAR in pain control are supported by their direct physical interaction. This finding provides the rationale to develop bifunctional drugs, opioid agonist coupled to NMDAR antagonist, to act exclusively on those NMDARs associated with MORs. Supported by FIS/PI08-0417 and FIS-PS09/00332 grants from the ISCIII, MICINN.

O23-02

BLOCKADE OF SIGMA-1 RECEPTORS PREVENTS PACLITAXEL-INDUCED NEUROPATHIC PAIN AND SENSORY-NERVE MITOCHONDRIAL ABNORMALITIES IN MICE

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Paclitaxel is a widely used antineoplastic drug that frequently produces neuropathic pain. It has been hypothesized that paclitaxel-evoked neuropathy is due to a toxic effect on axonal mitochondria that impairs the axons energy supply. Sigma-1 receptors (S1-R) have a key role in paclitaxel-induced neuropathic pain, but it is unknown whether they are related to these mitochondrial abnormalities. Paclitaxel 2 mg/kg was administered i.p. once per day during five consecutive days, to wild type (WT) and S1-R knockout (KO) mice. In separate experiments, WT mice were treated with the S1-R antagonist BD-1063 (32 mg/kg, s.c.), 30 min before each paclitaxel injection. Cold-allodynia (acetone test) and mechanical-allodynia (electronic Von Frey test) were tested previously to these treatment and 10 and 28 days after them. Following behavioural tests, mice were transcardially perfused and portions of saphenous nerves were removed and appropriately processed for electron microscopy study. WT mice treated with paclitaxel developed cold- and mechanical-allodynia and a significant increase in the frequency of swollen and vacuolated mitochondria in myelinated fibers (A-Beta > A-Delta), only on day 10 post-treatment. However, there was no evidence of paclitaxel-induced axonal degeneration, myelin damage, or microtubule changes. WT mice pretreated with BD-1063 and S1-R KO mice did not develop neuropathic pain or significant mitochondrial abnormalities after paclitaxel treatment. These results suggest that activation of S1-R is required for the appearance of paclitaxel-induced pain and sensory nerve mitochondrial damage. Therefore, S1-R antagonists might have therapeutic value for prevention of paclitaxel-induced neuropathic pain. Supported by Junta-Andalucía (CTS-109), MEC (SAF2006-06122), Laboratorios Esteve and MEC-FPU

O24-02

POTENTIAL ANTIPSORIATIC EFFECT OF A BENZOTHIOPHENE Γ -HYDROXYBUTENOLIDE DERIVATIVE: IN VITRO AND IN VIVO STUDY

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Introduction: In a previous study we reported the inhibitory effect of 4-benzo[b]thiophen-2-yl-3-bromo-5-hydroxy-5H-furan-2-one (BTH) on NFκB activation and on TNFα and IL-8 release in stimulated primary human keratinocytes. In the present study, we evaluated the effect of BTH in TPA-stimulated psoriatic human keratinocytes, as well as in the TPA-induced hyperplasia murine skin model, which presents some similarities with psoriatic lesions.

Methods: Psoriatic human keratinocytes were isolated from patients with active chronic plaque psoriasis from the Hospital La Plana (Vila-real). All patients gave informed written consent. After 7 h-stimulation with TPA (1 μg/ml), TNFα and IL-8 levels were determined by ELISA. Hyperplasia was induced in shaved backs of females Swiss mice by topical repeated administration for 3 days of TPA (2 nmol/site). BTH was applied to the same area 1 h before stimulus. After sacrifice, skin biopsies were obtained to determine edema, myeloperoxidase activity and TNFα/CXCL-1 release.

Results and Conclusions: Stimulated keratinocytes released 75.5 ± 10.3 pg/ml of TNFα and 616.5 ± 109.3 pg/ml of IL-8. BTH inhibited the release of these two cytokines in a concentration-dependent manner within the micromolar range. Topical application of BTH (400 μg/site) significantly reduced edema (66.8%) and neutrophilic infiltration (54.9%), as well as TNFα (1313 ± 126 pg/ml vs. 1829 ± 91 pg/ml in control group) and CXCL-1 (93 ± 11 pg/ml vs. 143 ± 9 pg/ml in control group) in tissue homogenates. Our results show the ability of BTH to inhibit several key biomarkers up-regulated in inflammatory skin diseases such as psoriasis.

O25-02

HISTOLOGICAL DAMAGE CORRELATES WITH THE NUMBER OF HIF-2 POSITIVE CELLS IN THE LAMINA PROPRIA OF PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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Introduction: Epithelial barrier function is impaired in the inflammatory bowel disease (IBD). Hypoxia and cytokines, key features of inflammation, modulate the activity of hypoxia inducible factor (HIF), a transcription factor related to the induction of genes involved in mucosal healing. We aim to determine the pattern of HIF expression in the intestinal mucosa of IBD patients.

Patients and Methods: Both damaged and non-damaged mucosa from patients with ulcerative colitis (UC) and Crohn disease (CD) were obtained. Paraffin embedded tissues were used for histological analysis (score 1–4) and ki67 immunostaining (cellular proliferation, score 1–4). Presence of macrophages, HIF-1α and HIF-2α were analyzed by immunohistochemistry.

Results: Cellular proliferation was increased in damaged mucosa of UC (2.8 ± 0.3) and CD (1.7 ± 0.3) compared with non-damaged mucosa (1.3 ± 0.3 and 1 ± 0.05 , respectively). A correlation was observed between mucosal damage and the number of macrophages in the lamina propria; the Spearman correlation coefficient was $r = 0.6949$ ($P = 0.02$, $n = 11$) for UC and $r = 0.73$ ($P = 0.046$, $n = 8$) for CD. HIF-1α immunostaining was just observed in few epithelial cells. In contrast an increased HIF-2α immunostaining was observed in cells of the lamina propria of the damaged area of UC and CD. A correlation was observed between mucosal damage and the number of HIF-2 positive cells; the Spearman correlation coefficient was $r = 0.842$ ($P = 0.02$, $n = 7$) for UC and $r = 0.785$ ($P = 0.027$, $n = 8$) for CD.

Conclusion: The number of HIF-2α positive cells in the lamina propria increases with the severity of disease in the intestinal mucosa of patients with IBD.

O26-02

ENHANCEMENT OF MORPHINE-INDUCED ANALGESIA AGAINST MECHANICAL NOCICEPTIVE STIMULI BY SIGMA-1 RECEPTOR BLOCKADE: STUDIES WITH A SELECTIVE SIGMA-1 ANTAGONISTS

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There is a tonically active sigma-1 (σ1) endogenous system in the CNS, which inhibits opioid-induced analgesia against thermal nociceptive-stimuli. However, it is unknown whether a similar interaction happens in peripheral nociceptors and against mechanical nociceptive-stimuli. Pressure (450 g) was applied to hind-paws of wild-type and σ1-knockout (σ1-KO) mice with an Analgesy-Meter (Ugo Basile, Italia) until the mouse showed a struggling behavior or 60 s had passed (cut off time). Morphine (0.5–64 mg/kg, s.c.) was administered 30 min before evaluation and BD-1063 (1–32 mg/kg, s.c.) 5 min before morphine. The analgesic effect of intraplantar (i.pl.) administration of morphine (50–200 μg/20 μl) and morphine + BD-1063 (200 μg/20 μl) was measured 5 min after injection. The density of μ opioid receptors was measured with [3H]-DAMGO (0.25–45 nM) saturation binding assays in brain synaptosomes. Morphine s.c. produced a dose-dependent antinociception (in both paws) with a higher potency and efficacy in σ1-KO than in wild-type mice. Morphine i.pl. also produced a dose-dependent antinociception but only in the injected paw and only in σ1-KO mice. [3H]-DAMGO binding characteristics were the same in WT and σ1-KO mice. In wild-type mice, but not in σ1-KO, the analgesic effect of morphine was dose-dependently potentiated by BD-1063 administration. The potentiation happens in both paws when drugs were s.c. injected, but only in the injected paw after i.pl. administration. These results suggest that genetic inactivation and pharmacological antagonism of σ1 receptors in peripheral nociceptors increase the analgesic effect of morphine.

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O27-02

CARBON MONOXIDE SYNTHESIZED BY HEME OXYGENASE 1 ENHANCED THE EFFECTS AND EXPRESSION OF M-OPIOID RECEPTORS DURING NEUROPATHIC PAIN IN MICE

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Introduction: μ-opioid receptor (MOR) agonists attenuate neuropathic pain with low efficiency as a consequence the research of new therapeutic strategies to improve their efficacy is necessary. We investigated if carbon monoxide, synthesized by heme oxygenase-1 (HO-1), could modulate the effects and expression of MOR during neuropathic pain.

Material and Methods: In C57BL/6 mice, at 10 days after chronic constriction of sciatic nerve induction of neuropathic pain, we evaluated the antiallodynic and antihyperalgesic effects of the subplantar administration of a MOR agonist (morphine; 50 μg) and the intraperitoneal administration of a carbon monoxide releasing molecule-2 (CORM-2; 5 mg/kg/twice a day) or a HO-1 expression inducer (cobalt protoporphyrin IX, CoPP; 2.5 mg/kg/twice a day) alone or combined. The protein levels of MOR in dorsal root ganglia from nerve-injured mice treated with CORM-2 or CoPP were also assessed.

Results: One day treatment with CORM-2 or 2 days with CoPP reduced the principal neuropathic pain symptoms and significantly enhanced the local mechanical antiallodynic, thermal antihyperalgesic and thermal antiallodynic effects produced by a subanalgesic dose of

morphine. This study also showed that both CORM-2 and CoPP treatments enhanced the expression of MOR in sciatic nerve-injured mice.

Conclusion: These results indicate that carbon monoxide synthesized by HO-1, increases the effects and expression of MOR after nerve injury and suggest that the local administration of opioids with CORM-2 or CoPP might represent a useful new strategy for the treatment of neuropathic pain.

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O28-02

THE ANTINOCICEPTIVE EFFECTS OF JWH-015 IN CHRONIC INFLAMMATORY PAIN ARE PRODUCED BY THE NITRIC OXIDE-CGMP-PKG-KATP PATHWAY ACTIVATION MEDIATED BY OPIOIDS

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Introduction: We investigated if the peripheral nitric oxide-cGMP-protein kinase G (PKG)-ATP-sensitive K⁺ (KATP) channels pathway, triggered by neuronal nitric oxide synthase (NOS1) and modulated by opioids, participates in the local antinociceptive effects produced by a cannabinoid-2 receptor (CB2R) agonist (JWH-015) during chronic inflammatory pain.

Material and Methods: In wild type and NOS1-KO mice, at 10 days after the subplantar administration of complete Freund's adjuvant (CFA), we evaluated the antinociceptive effects produced by the subplantar administration of JWH-015 and their reversion by the local co-administration with specific CB2R (AM630), peripheral opioid receptor (naloxone methiodide, NX-ME) or CB1R (AM251) antagonists. Expression of CB2R and NOS1, and the antinociceptive effects produced by JWH-015 combined with selective L-guanylate cyclase (ODQ) or PKG (Rp-8-pCPT-cGMPs) inhibitors or a KATP blocker (glibenclamide), were assessed.

Results: The local administration of JWH-015 dose-dependently inhibited the mechanical and thermal hypersensitivity induced by CFA which effects were reversed with AM630 or NX-ME, but not AM251. Inflammatory pain increased the expression of NOS1, but not CB2R. The antinociceptive effects of JWH-015 were absent in NOS1-KO mice and diminished by their co-administration with ODQ, Rp-8-pCPT-cGMPs or glibenclamide.

Conclusions: The peripheral antinociceptive effects of JWH-015 during chronic inflammatory pain are mainly produced by the local activation of nitric oxide-cGMP-PKG-KATP pathway, triggered by NOS1 and mediated by endogenous opioids, suggesting that activation of this pathway might be an interesting therapeutic target for the treatment of chronic inflammatory pain with cannabinoids.

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O29-02

TACKLING THE MGLUR4 PAIN TARGET BY COMBINED CHEMICAL, PHARMACOLOGICAL AND COMPUTATIONAL APPROACHES

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Introduction: Recent studies have shown that positive allosteric modulators (PAMs) of mGluR4 yield beneficial effects in neuropathic and

inflammatory pain (1,2). However, only very few compounds acting on this receptor have been identified so far. Noticeably, chirality plays a role in receptor activation. The aim of our work was to provide insight on the structural and pharmacological determinants of PAM mechanism both from the ligand and the receptor sides.

Material and Methods: The chiral VU0155041 PAM was used as a reference. To identify the active and inactive enantiomers, asymmetric synthesis was performed (3). To restrict the conformational freedom of the cyclohexane moiety a C=C bond was inserted in particular positions of the ring. The receptor was cotransfected with a chimeric Gq/i-protein, which couples to the phospholipase C pathway, thus allowing monitoring of receptor activity by measurement of Ca²⁺ release. High-level quantum chemical calculations were performed on ligand compounds to characterize potential active ligand conformations. Homology modeling techniques were used to construct a model for the transmembrane domain of mGluR4. A selected set of mutations were carried out to identify the protein residues involved in PAM mechanism.

Results: From pharmacological data of VU0155041 and derivative compounds, structural determinants of activity have been found for both the ligand and the receptor binding site.

Conclusion: Accurate analysis of chiral and conformational properties of PAMs are fundamental for pharmacological characterization of receptor activity and drug design.

References:

1. Pain 2008; 137: 112–124.
2. Neuroreport. 2011; 22(5):244–8.
3. Chem Med Chem 2011; 6(1):131–40.

O30-02

IMPROVED CLINICAL PROGNOSIS AND CHEMOKINE LEVES IN THE SINOVIIUM OF OSTEOARTHRITIC PATIENTS TREATED WITH CONDROITIN SULFATE BUT NOT WITH PARACETAMOL

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Background: Osteoarthritis (OA) is a chronic joint disorder characterized by a slow progressive degeneration of articular cartilage, subchondral bone alteration and variable secondary synovial inflammation. Although OA was defined as a noninflammatory arthropathy, several inflammatory mediators have been implicated in OA. Objective: To investigate the clinical consequences and the levels of chemokines in OA patients treated either with chondroitin sulphate (CS) or paracetamol.

Methods: Sixty to twenty OA patients were treated with CS (800 mg/day) or paracetamol (4 g/day). Clinical examination of the synovia (measurement of the overflow, hypertrophy and vascularisation) and functional study of the articulation (with Lequesne and EVA index) were performed. The levels of CXCL16, fractalkine/CX₃CL1, MCP-1/CCL-2, RANTES/CCL5 or GRO- α /CXCL1 were determined by ELISA in plasma, synovium and urine samples collected at baseline and after 1, 6 and 9 months of treatment.

Results: Improvements in the clinical signs of inflammation were detected after 6–9 months CS treatment of OA patients. These effects were accompanied by reductions in the synovial levels of CXCL16, fractalkine or MCP-1. In plasma and urine samples, only MCP-1 concentration was diminished by CS administration. When OA patients were treated with paracetamol, neither clinical scores of inflammation were improved nor chemokine concentrations modified.

Conclusions: These results suggest that CS sulphate may represent an adequate drug to reduce the inflammation associated to the OA process. This work was supported by grants SAF2008-03477, PI/08/1875, RIER RD08/0075/0016, from Spanish Ministry of Science and Innovation, Carlos III Health Institute, Spanish Ministry of Health and other grants from Generalitat Valenciana.

NEUROPSYCHOPHARMACOLOGY

O031-03

BEHAVIOURAL EFFECTS OF HINT1 DEFICIENCY: A MODEL FOR THE MANIC POLE OF BIPOLAR DISORDER

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Bipolar disorder (BPD) is a chronic illness which includes alternate episodes of depression and mania. The paucity of appropriate animal models of BPD hinders the research of its pathophysiology as well as the development of new treatments for this neural disease. A number of genes, molecules and pathways have been implicated in BPD, being HINT1 one the gene targets so far identified. Indeed, a human post-mortem analysis revealed a reduced HINT1 protein expression in prefrontal cortex of bipolar patients. Therefore, the present study comparatively analyzes the performance of HINT1^{-/-} (KO) and HINT1^{+/+} (WT controls) mice in a battery of tests that have better validation for manic-like behaviours (1). Those tests include spontaneous activity, sweet solution preference, light/dark box, resident-intruder, forced-swim and apomorphine induced hyperactivity. The effects of mood stabilizers (valproate, lithium) and antidepressants (imipramine and citalopram) were evaluated in these behaviour tests. The results indicate that, compared to HINT1^{+/+} controls, HINT1^{-/-} mice exhibit higher level of aggression, they spend more time in the light compartment, a lower immobility time in the forced-swim test, and an intense response to apomorphine. Administration of lithium and valproate, but not that of imipramine or citalopram, attenuated these manic-like behaviours. Our results suggest that HINT1^{-/-} mice reproduce different domains of mania and, what is more relevant, they provide a suitable substrate to study the molecular basis of the BPD. (Einat H. J. *Psychopharmacol* 2006; 20:714–22).

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O032-03

NEUROCHEMICAL PROFILE OF NEW BETA-KETO AMPHETAMINES: MEPHEDRONE AND BUTYLONE

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Mephedrone (Mef) and butylone (But), are cathinone derivatives that seems to elicit stimulant effects in humans similar to cocaine or MDMA. To our knowledge there is not any demonstration of the mechanism of action of these drugs. Mef and But were synthesized by us. Uptake experiments of were performed in isolated rat brain synaptosomes from striatum and cortex. Binding experiments were carried out in membrane preparations from the same rat brain areas. Mef and But inhibit dopamine and serotonin uptake (IC₅₀ values of 0.79 ± 0.07 μM; 0.31 ± 0.08 μM for Mef and 2.01 ± 0.18 μM; 0.68 ± 0.13 μM for But). Mef and But interact with the dopamine transporter (K_i: 1.53 ± 0.47 μM for Mef and 0.44 ± 0.04 μM for But), measured as inhibition of [3H]WIN 35428 binding and also with the serotonin transporter (K_i: 17.55 ± 0.38 μM, for Mef and 3.10 ± 0.12 μM for But) measured as inhibition of [3H]paroxetine binding. Mef and But also displayed a great affinity for 5-HT₂ than for D₂ receptors. These results provide evidence, for the first time, that Mef and But interact directly with both dopamine and serotonin transporters increasing neurotransmitter concentration at synapses and can induce a psychostimulant effect. Mef showed a great affinity for dopamine than for serotonin transporter and But displayed a similar affinity for both transporters. This work was supported by grants from Plan Nacional sobre Drogas (2008/003; 2010/005), Ministerio de Ciencia e Innovación (SAF2010-15948) and Generalitat de Catalunya (SGR977).

O033-03

PLASMALEMAL AND MITOCHONDRIAL SODIUM-CALCIUM EXCHANGERS AS TARGETS FOR POTENTIAL NEUROPROTECTIVE DRUGS

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Common to neurodegenerative diseases is an alteration of Ca²⁺ homeostasis in neurons. The cytosolic Ca²⁺ elevations in response to cell activation mediate a great number of neurophysiological effects. However, these [Ca²⁺]_c transients must be regulated, because beyond a certain threshold level, an elevation of the [Ca²⁺]_c may become neurotoxic. In this regulation to maintain the [Ca²⁺]_c within physiological levels, the plasmalemmal (pNCX) and mitochondrial (mNCX) Na⁺/Ca²⁺ exchangers play critical roles. We have tested the hypothesis that the pharmacological regulation of those ion transporters may lead to neuroprotection on neurons made vulnerable by a stress based on Na⁺ and Ca²⁺ overload elicited by veratridine. The rat hippocampal slices were subjected to stress with veratridine (30 μM) and treated with CGP37157 (3 and 30 μM), a mNCX blocker, and with KB-R7943 (1, 3 and 10 μM), a pNCX blocker. Compound CGP37157 significantly protects rat hippocampal slice neurons against veratridine neurotoxicity, in a concentration dependent manner. Compound KB-R7943 also produced a concentration dependent significant protection. We are now trying to combine both blockers at various concentrations to test the hypothesis that simultaneous regulation of pNCX and mNCX constitutes a novel strategy for enhanced neuroprotection. The outcome of those experiments will surely inspire the synthesis of multi-target ligand compounds with potential neuroprotective properties. These compounds may have the capacity of rescuing from death the vulnerable neurons of degenerative diseases and stroke.

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O034-03

THE RESPONSES TO SUSTAINED ACETYLCHOLINE OF C57 MOUSE ADRENAL CHROMAFFIN CELLS

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The effects of stimulation with acetylcholine (ACh) of C57 mouse chromaffin cells are unknown. This mouse strain is widely used to make transgenic disease models and thus, we felt interest to analyse the effects of long ACh pulses on excitability, vesicle movement and quantal release of catecholamine. Patch-clamp, amperometric and TIRF microscopy techniques were combined, to explore the effects of ACh on primary cultures of C57 mouse adrenal medullary chromaffin cells. One-min pulses of 100 μM ACh elicited a burst of action potentials (APs) followed by sustained depolarisation. This is accompanied by an inward current that inactivates to a sustained small plateau. ACh depolarisation may be linked to inhibition by ACh of Ca²⁺-dependent outward K⁺ currents. ACh also elicited a burst of quantal secretory spikes that inactivated to a smaller rate on single-vesicle release. Under TIRF microscopy, vesicle movements paralleled those two phases of quantal secretion. Prolonged stimulation with ACh caused a rapid followed by slow phases of quantal catecholamine release that can be explained by the sequential activation

of inactivating nicotinic receptors, followed by non-inactivating muscarinic receptors. These results on control C57 mice will serve as a template to compare future results in chromaffin cells of transgenic models of Alzheimer's disease and amyotrophic lateral sclerosis.

O035-03

PHARMACOLOGICAL VALIDATION IN A NEW MODEL OF EPILEPSY: THE HAMSTER GASH:SAL

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The Hamster GASH:Sal has been developed at the University of Salamanca as a new model of audiogenic epilepsy. Up to this day, the genetically determined seizure response of this Hamster has not yet been evaluated using antiepileptic drugs. Here, we characterized the tonic/clonic seizures of the Hamster GASH:Sal after administration of classic (Phenobarbital and Valproate) and new generation (Levetiracetam) antiepileptic drugs, assessing the anticonvulsant activity and the active plasmatic concentration of the drugs. Simultaneously, we characterized the ethological changes of the seizures induced by drugs. Seizures were induced by an auditory stimulus. HPLC determination of plasma levels is performed after administration of maximal doses of the drugs. To determine the anticonvulsive activity, a dose-effect curve was performed by injecting intraperitoneally four increasing doses of each antiepileptic drug before to induce the seizure. Video-records of the behavioral patterns were done during each seizure using the program ETHOMAX (García-Cairasco, 1983). The ED50 values for control of seizures in this model rose in a dose-dependent manner. Levetiracetam controls the tonic-clonic convulsions since the minimal dose and stop the 80% of the crisis at 50 mg/kg. Phenobarbital stop the seizure at 15 mg/Kg. Valproate generated the maximal effect with the higher dose only. The anticonvulsive effects of Levetiracetam are higher than those observed with Phenobarbital and Valproate in the Hamster GASH:Sal model. The anticonvulsive effect is dose-dependent, and it is characterized by a reduction of tonic-clonic phase, and a longer duration of wild running and post-ictal phase.

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O036-03

ROLE OF OREXIN RECEPTOR TYPE 1 IN THE ACTIVATION OF THE BRAIN STRESS SYSTEMS DURING MORPHINE WITHDRAWAL

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The hypothalamus-pituitary-adrenal (HPA) axis and the extrahypothalamic brain stress system, also called extended amygdala [which includes the nucleus accumbens-shell, the bed nucleus of the stria terminalis (BNST) and the central nucleus of the amygdala (CeA)] are key elements of the neural circuitry that regulates the negative states and the increased drug seeking during abstinence from chronic drug exposure. Orexins are recently discovered neuropeptides that have been hypothesized to modulate the extended amygdala activity and to

contribute to the negative emotional state associated with dependence. In this study we examined the possible involvement of orexin receptor 1 (OX1R) on morphine withdrawal-induced activation of brain stress systems. The OX1R antagonist SB334867 was used to evaluate if orexins activity is related to alterations in HPA axis and extended amygdala in rats dependent on morphine. Male Wistar rats were implanted with two pellets of morphine or placebo. Seven days later, rats received SB334867 or vehicle, and were injected with saline or naloxone 20 min later. CRF and c-Fos expression were evaluated by immunohistochemistry, and plasma corticosterone levels were measured by RIA. SB334867 administration decreased c-Fos expression in the CeA CRF neurons during morphine withdrawal. Morphine-dependent rats administered with SB334867 showed higher plasma corticosterone responses to naloxone administration than the morphine-dependent group receiving vehicle. Presents results indicate that OXA are implicated, through OX1R, in the CeA activation during morphine withdrawal, and may suggest the involvement of OX2R in morphine withdrawal-induced activation of CRF neurons from the BNST and PVN, and HPA axis hyperactivity.

O037-03

INCREASED TAU PHOSPHORYLATION IN THE CORTEX OF PPAR β / δ -DEFICIENT MICE

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Introduction: The main histological findings in Alzheimer disease (AD) are the accumulation of extracellular senile plaques consisting mainly of β -amyloid, which is derived from amyloid precursor protein through cleavage by two secretases, the β -secretase 1 (BACE 1) and the δ -secretase, and the intracellular accumulation of neurofibrillary tangles, which consist of tau oligomers and abnormally phosphorylated form of tau protein. Several kinases are able to phosphorylate tau protein, including Cdk 5, ERK 1/2, and GSK-3 β . Little is known about the role Peroxisome Proliferator Activated Receptor (PPAR) β / δ in AD. Here we examined the effect of PPAR β / δ deficiency on BACE1 expression and tau phosphorylation in cortex.

Material and Methods: Cortex were obtained from five-months-old male PPAR β / δ knockout mice and control mice (PPAR β / δ ^{+/+}, wild-type, n = 5) with the same genetic background (C57BL/6X129/SV).

Results: PPAR β / δ -null mice showed increased mRNA levels of BACE1 (2-fold induction, P < 0.05) in cortex compared to wild-type mice. In addition, the expression of IL-6 (2.2-fold induction, P < 0.05) and RAGE (receptor for advanced glycation end products) (1.7-fold induction, P < 0.05) was also increased. Interestingly, PPAR β / δ -null mice showed increased phospho(Ser¹⁹⁹)-tau levels in cortex compared to wild-type mice. This increase was accompanied by enhanced protein levels of several kinases involved in its phosphorylation, such as Cdk5 and ERK1/2. In contrast, the levels of phospho(Ser³)-GSK-3 β were increased, suggesting that this kinase did not contribute to the increase in tau phosphorylation.

Conclusion: These findings suggest that PPAR β / δ might regulate BACE1 expression and tau phosphorylation in cortex.

This work was supported by grant SAF2009-06939 (MICINN).

NATURAL PRODUCTS

O38-04

SHIKONIN PROMOTES WOUND HEALING THROUGH STAT3 ACTIVATION IN THE INTESTINAL EPITHELIAL CELL LINE IEC-18

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Introduction: The intestinal epithelial cell barrier plays an important role in maintaining mucosal immune homeostasis. STAT3 activation in intestinal epithelial cells protects from colitis promoting wound healing. In our search for anti-inflammatory agents from natural products we found that shikonin ameliorates dextran sulphate sodium-induced acute colitis through the inhibition in immune cells of the pro-inflammatory nuclear transcription factors NF- κ B and STAT3. Since clinical studies have already proved that shikonin modulates wound healing in a broad range of skin ulcers, in this study we have investigated whether shikonin has this same healing ability in intestinal epithelial cells and, therefore, contributes with this mechanism to the improvement of the evolution of experimental colitis.

Material and Methods: IEC-18 cells were plated at a concentration of 3×10^5 cells/ml and monolayers were either wounded with a razor blade or left untouched. Cells were incubated with or without shikonin 1 μ M, as described previously. Cells were collected and STAT3 and NF- κ B activation were determined by Western blot.

Results and Conclusions: Shikonin significantly induced wound healing in IEC-18 cells without affecting proliferation through a mechanism which promotes STAT3 activation and having no effect on NF- κ B. The increase in epithelial cell monolayer restitution in vitro suggests that shikonin might also stimulate the migration of epithelial cells to ulcerative areas in the colitis setting. The reduced pro-inflammatory insults would subsequently decrease the severity and reduce the duration of the disease.

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O39-04

LOOKING FOR THE ANTI-INFLAMMATORY ACTIVITY OF APOCYNIN AND ITS METABOLITE DIAPOCYNIN ON LPS-STIMULATED RAW264.7 MACROPHAGES

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Introduction: Ulcerative colitis is characterized by extensive gut inflammation and tissue injury enhanced by the production of reactive oxygen species (ROS) largely generated by membrane-associated NADPH oxidase. Apocynin is an efficient inhibitor of this enzyme although it is a prodrug that is converted to its dimer diapocynin, which is more efficient than apocynin itself. Recently, we have demonstrated the protective effect of apocynin in an experimental DSS-induced colitis model. In order to compare the molecular mechanisms of both compounds, in this work we have studied the effect on several inflammatory mediators in LPS-stimulated RAW264.7 macrophages.

Material and Methods: Diapocynin was synthesized and its purity was analyzed by ¹H-NMR and LC-MS. RAW264.7 cells were treated with apocynin and diapocynin (100 and 400 μ M) and stimulated with LPS (1 μ g/ml). Intracellular ROS was evaluated by non-polar DCFH-DA dye. NO production was monitored by the Griess reaction. The levels of TNF- α , IL-6 and IL-1 β were measured by ELISA and iNOS and COX-2 expression were determined by Western blot.

Results and Conclusions: Apocynin and diapocynin have anti-inflammatory effects but there are some differences. At the lower concentration,

diapocynin was more effective than apocynin and reduced by 59% ROS production, suppressed the LPS-mediated TNF- α , IL-6 and IL-1 β production (over 80% each) and inhibited iNOS expression by 55%. Apocynin, at the same concentration, only inhibited significantly the IL-6 production by 57%. These results reinforce the importance of the metabolism of apocynin for its biological effects.

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O40-04

LONG-CHAIN FATTY ALCOHOLS FROM EVENING PRIMROSE OIL MODULATE THE RELEASE OF PROINFLAMMATORY MEDIATORS

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Introduction: Evening primrose oil (EPO) is a natural product extracted by cold-pressed from *Oenothera biennis* L. seeds. The unsaponifiable matter (UM) of this oil is an important source of interesting minor compounds, like long-chain fatty alcohols (LCFAs), which are present free or as part of waxes.

Material and Methods: LCFAs were isolated from UM of EPO (CEE/2568/91), and the composition was identified and quantified by GC-MS. The ability of LCFAs from EPO to inhibit the release of different proinflammatory mediators in vitro was investigated using peritoneal murine macrophages stimulated with lipopolysaccharide. Cell viability was assayed by MTT test. The generation of nitrites was also quantified by Griess reaction method. COX-2 and iNOs changes in protein expression were detected by western blotting.

Results: The major components of LCFAs from EPO were tetracosanol (31.59%) and hexacosanol (36.52%). LCFAs significantly and dose-dependently decreased nitric oxide production. Western blot analysis showed that nitric oxide reduction was consequence of the inhibition of inducible nitric oxide synthetase expression.

Conclusion: These results showed that LCFAs may have an inhibitory effect on some mediators involved in the inflammatory damage development. LCFAs show a new potential value as functional component of Evening Primrose oil.

O41-04

SUPPRESSIVE EFFECTS ON PRO-INFLAMMATORY MEDIATORS BY OLEUROPEIN AND HYDROXYTYROSOL IN BALB/C PERITONEAL MACROPHAGES

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Introduction: Secoiridoid oleuropein and its derivative, hydroxytyrosol appear abundantly in fruits and leaves of *Olea europea*. Oleuropein is metabolized in the intestine to hydroxytyrosol. These molecules have many beneficial effects, such as antioxidant, antitumoral and anti-inflammatory activities. In previous studies, we showed the protective effect of oleuropein in a dextran sulphate sodium-induced colitis in mice. In order to investigate the possible mechanism, we studied the anti-inflammatory effect of these products on BALB/c mice peritoneal macrophages stimulated with lipopolysaccharide.

Material and Methods: BALB/c mice were injected intraperitoneally 2 ml of 3% thyoglycollate medium. After 4 days, mice were sacrificed and macrophages were isolated and seeded onto 96- and 24-well plates and pre-treated with oleuropein and hydroxytyrosol (50 and 100 μ M).

One hour later, macrophages were stimulated with LPS for 24 h. Cytokines IL-1 β , IL-6, TNF- α and IFN- γ were determined by ELISA in the supernatants and COX-2 and iNOS levels were measured by Western blot.

Results and Conclusion: Hydroxytyrosol (100 μ M) reduced IL-1 β (90%), IL-6 (50%), TNF- α (30%) and IFN- γ (70%) production, nitric oxide production (90%) and iNOS expression (50%). Moreover, oleuropein (100 μ M) reduces IL-1 β (64%), IL-6 (40%), nitric oxide production (77%) and COX-2 expression (20%). In conclusion, both reduced the proinflammatory mediators in stimulated murine peritoneal macrophages.

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O42-04

ANTI-OXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF UNSAPONIFIABLE FRACTION OF EXTRA VIRGIN OLIVE OIL IN MURINE PERITONEAL MACROPHAGES

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Background: Recent studies have confirmed that regular extra virgin olive oil (EVOO) consumption, is effective in the prevention and treatment of pathologies related to the oxidative stress, chronic inflammation and the immune system. Traditionally the beneficial effects of VOO have been attributed to its monounsaturated fatty acids. Unsaponifiable fraction (UF) is constituted by a wide number of compounds of high biological value. Aims: To study the anti-inflammatory and antioxidant activities of UF of EVOO in lipopolysaccharide (LPS)-stimulated murine peritoneal macrophages.

Methods: The isolation of UF fraction was carried out by means of liquid-liquid partition with diethyl ether followed by washings with water. The quantitative determination of unsaponifiable components, was carried out by HPLC. Cell viability was assayed by SRB test. Antioxidant activity was measured by DPPH free radical scavenging method. The generation of nitrites was also quantified. UF induced changes in protein expression of COX-2, iNOS and MAPKs pathways were detected by western blotting.

Results: Main compounds present in the UF were squalene, sterols, triterpenic and alifatic alcohols. The treatment with UF produced no changes in cell viability. UF showed a significant antioxidant activity in DPPH test. Moreover, the generation of nitrites was significantly prevented in UF treated cells. Cell treatment with different μ g/ml concentration of UF induced a down-regulation of pro-inflammatory enzymes COX-2 and iNOS. Besides UF-treated macrophages showed significant decreases in the phosphorylation of MAPKs protein expressions.

Conclusion: These preliminary data suggest that UF show significant antioxidant and anti-inflammatory activities in murine peritoneal macrophages.

O43-04

Ω -3 POLYUNSATURATED FATTY ACIDS FROM MICROALGAE INHIBIT TNF- α PRODUCTION IN THP-1 CELLS AND REDUCE INTESTINAL INFLAMMATION IN AN ANIMAL STUDY

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Background: The marine environment represents a vast source for isolation of new microbes, as microalgae, that are potent producers of

bioactive secondary metabolites. As photoautotrophics, their simple growth requirements make them attractive for bioprocesses aimed at producing high added-value compounds that are in large demand by the pharmaceutical market. Actually, fatty acids have taken a very important role due to its immunomodulatory and antioxidant capacity, with ability to control cancer and inflammatory processes.

Objetive: To study the *in vitro* and *in vivo* anti-inflammatory properties of three ω -3 PUFAs (GF-A,GF-B,GF-C), isolated from a Chlorophyceae (*Nannochloris spp.*), and which have been identified as oxylipins.

Results: The *in vitro* activity was tested on THP-1 cells (human monocytic leukemia cell line), by quantification of inflammatory cytokine production. The results showed high inhibition of TNF- α production for GF-A, GF-B, and GF-C. Otherwise, we assayed the ability of oxylipins to interfere with the NF- κ B signaling pathway (HT-29 human colon cancer cells) by an immunofluorescence technique. We observed an important reduction of NF κ B translocation into the nucleus, and, conversely, outstanding PPAR-gamma nuclear localization. The *in vivo* activity was studied in a model of acute colitis by trinitrobenzenesulfonic acid (TNBS) in Wistar rats. The oral supply of lyophilized from *Nannochloris* caused a marked amelioration of wasting (weight loss, diarrhoea), macroscopic (ulceration surface, adhesences, colon weight/length ratio) and histopathologic (neutrophilic infiltration, necrosis) signs of damage in animals treated vs control-TNBS animals.

Conclusions: oxylipins obtained of microalgae are potent anti-inflammatory molecules, with potential interest to treatment inflammatory process including IBD.

O44-04

CHEMICAL HYDROXYL RADICAL PROTECTION OF HYDROXYTYROSYL ETHER MODIFIES ITS ANTIOXIDANT AND NEUROPROTECTIVE EFFECTS

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Hydroxytyrosol has neuroprotective effect when it is proved in rat brain slices during an hypoxia reoxygenation model. The confirmation of the presence of the free hydroxyl radicals in the antioxidant and/or neuroprotective effects of this compound has not been demonstrated previously. The aim of this study is to analyze the antioxidant and neuroprotective effects in rat brain slices of some phenolic compounds with different free OH groups. An *in vitro* (concentrations 1–1000 μ M) and an *ex vivo* (10 and 20 mg/kg/day for 7 days p.o.) studies were carried out of each compounds: hydroxytyrosyl-ether (two free OH groups), tyrosol-ether and homovanilic-ether (both with one free OH group) and metiliden-ether (with none free OH groups). TBARS and LDH efflux in rat brain slices subjected to an hypoxia and reoxygenation model were determined. TBARS production in rat brain slices was inhibited in a concentration-dependent manner (IC₅₀, μ M): 7.68 for hydroxytyrosyl-ether, 420 for tyrosol-ether, 105 for homovanilic-ether and >1000 (no effect) for metiliden-ether. LDH efflux was inhibited in a concentration-dependent manner (IC₅₀, microM): 28.1 for hydroxytyrosyl-ether, 84.3 for tyrosol-ether, 216 for homovanilic-ether and 436 for metiliden-ether. After oral administration brain TBARS content was inhibited as follows: hydroxytyrosyl-ether 11.18%, tyrosol-ether 29.02%, homovanilic-ether 25.99% and metiliden-ether 47.65%. LDH efflux was reduced as follows: hydroxytyrosyl-ether 26.92%, tyrosol-ether 71.18%, homovanilic-ether 75.20% and metiliden-ether 67.25%.

Conclusion: these results demonstrate a relation between the neuroprotective effect and the number of free OH of the ether derivatives.

O45-04

ORAL ADMINISTRATION OF ALKYL HYDROXYTYROSYL ETHERS EXERTS A NEUROPROTECTIVE EFFECT.

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A neuroprotective effect of alkyl hydroxytyrosyl ethers in a model of hypoxia-reoxygenation in rat brain slices has been demonstrated 'in vitro'. The aim of the present study is to investigate the possible neuroprotective effect after the oral administration of these compounds. Six compounds were tested: hydroxytyrosol (HT), HT ethyl ether, HT butyl ether, HT hexyl ether, HT octyl ether and HT dodecyl ether (HT-DTE). Each compound was administered by gavage in dose of 20 mg/kg/day

for seven days (n = 10 rats per group). A control group without treatment was considered. After the hypoxia-reoxygenation model in rat brain slices 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 and nitrite/nitrate production. These compounds inhibited LDH efflux with respect to control group. This effects was more appreciated with the compounds with longer alkyl carbon chains: octil 31.98% and dodecil 57.54% inhibition respect to non-treated rats. The levels of IL-1 β were likewise modified over control. Octil and dodecil decrease it by 34.32% and 27.95% respectively. TBARS decreased 60% and 70.66% respectively.

Conclusion: oral administration of alkyl hydroxytyrosyl ethers exerts a neuroprotective effect, mainly octyl and dodecyl derivatives.

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TEACHING IN PHARMACOLOGY

O046-05

EXTRACURRICULAR ACTIVITY IN TEACHING PHARMACOLOGY: DIGITAL POSTERS CONTEST

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Pharmacology is a crucial discipline for nursing students. It is important that students appreciate pharmacological principles and are able to develop 'transferable skills' to apply them in the clinical practice and in health education programs. The purpose of the present work was to provide an opportunity for the students to link the nursing principles of pharmacology with a pharmacological programme education for the university community. For that purpose the teachers of pharmacology of the University of Alicante organized a digital poster contest promoting 'the rationale use of drugs'. The target students group involved second and third year students of the Nursing Degree. The posters were projected during a week, 8 h per day, at the general library and the classrooms buildings. Furthermore, the posters could be visualized in the intranet. The voting was accomplished with a survey between all the students of the University of Alicante through an online questionnaire self-administrated in the intranet. All category prizes were awarded with a book of pharmacology. A total of 13 posters were selected and 1000 votes were counted. The nursing students considered the activity to be very positive. On the other hand, the large amount of votes evidenced the usefulness of this initiative for health education programs at the University of Alicante. We consider that extracurricular activities allow students to get involved in a creative way and help to reach learning aims.

O047-05

INNOVATIONS IN PHARMACOLOGY EVALUATION: PILOT STUDY ON THE PARTICIPATION OF STUDENTS IN "FUNDAMENTOS DE FARMACOLOGÍA EN FISIOTERAPIA"

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The EHEA includes the assessment process within a dynamic framework of the teaching-learning process. The multiple-choice question format evaluates knowledge, although always within a passive context. As lecturers of pharmacology we aimed to encourage active student participation in their own assessment in order to foster lifelong learning skills. We invited students to participate by formulating their own questions including an explanation/reasoning of the correct answer. Once reviewed for accuracy and clarity the questions were made available before the exam, through our Virtual Campus, to all students regardless of whether or not they had made any contributions. Student participation was 10.4%. A total of 40 questions were received and 4 were selected for the examination (6.6%). All students who contributed questions passed the examination, obtaining an average mark of 7.5 ± 0.7 compared with 5.9 ± 0.8 obtained by those that did not. The students who participated in the initiative obtained better results than their colleagues. The proposal of questions with a defined aim entails a type of learning over and above the mere presence of the student in the class. This type of initiative allows the educators to carry out a self-assessment: an analysis of the questions allows us to identify the topics in need of special attention and also those considered most important by the students for the attainment of their professional competencies. Escuela de Enfermería, Fisioterapia y Podología, UCM.

O048-05

ASSESSMENT OF EVALUATION BY THE STUDENTS OF THE RELATIONSHIP BETWEEN CONTENTS OF THE PHARMACOLOGY AND PHARMACY SUBJECT AND LABOR MARKET REQUIREMENTS DURING THE LAST FOUR YEARS AT THE FACULTY OF VETERINARY MEDICINE OF MURCIA (SPAIN)

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Introduction: Pharmacology and Pharmacy is a subject taught in the Third year of the Veterinary Medicine Degree at the University of Murcia. The new degrees associated to the Bologna plan have brought implementation of new teaching methodologies nearer to the labor market for the student.

Material and Methods: During the last 4 years, students received an evaluation test of the Pharmacology and Pharmacy subject, where they had to assess through several items how close they felt that teaching they were receiving were going to be useful when they get real labor market. Six specific items were design for this purpose that could be rated between one (disagree with the proposition) and five (totally agree with the proposition). Descriptive statistics and frequency distribution test was done to check evolution of the items through the 4 years.

Results and Discussion: Data show a better assessment by the students from the second year of evaluation. Second year is the starting point to implement new teaching methodologies (pharmaceutical industry round tables, external veterinarians clinical sessions, power points availability, etc) to adapt this subject to the Bologna Process. Data of the rest of the years (3 and 4) show that the students keep a high rate assessments of the six items evaluated.

Conclusion: New methodologies introduced into the Pharmacology and Pharmacy subject teaching have been of great efficacy to get the student to know how important this subject is for their professional future, with a closer contact with the real labor world the imparted contents.

O049-05

THE USE OF CROSSWORDS PUZZLES AS AN INNOVATIVE TEACHING METHOD FOR CLINICAL PHARMACY

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Introduction: One type of puzzle games commonly used in the learning process is crossword puzzle. Pharmacy students were encouraged to take active part in these innovations within pilot plan as a frame on the European Space for Higher Education. AIMS: The aim of this study was to see the practically of using the crossword puzzle as an interactive teaching method in final year pharmacy students.

Material and Methods: Students of Pharmacy Degree at the University of Seville were divided into nine working groups. Teachers developed nine different crosswords containing 20 definitions related to various topics of clinical pharmacy and were administered to the student to solve at the same time. Development of crossword was simple using EclipseCrossword[®] software.

Results: Participant students were evaluated according to the number of solving definitions at the moment that the first student group finished solving the crossword. Later, unsolved points of crosswords were discussed in the classroom and solutions were presented. A feedback was taken from the student regarding to the use of this innovative approach. Working students enjoyed the competitions among their classmates, the full autonomy in solving the crosswords and learnt new concepts in clinical pharmacy. It showed that using such exercise stimulated and activated the interactive learning among the students reflected by their positive attitude and response. Many students found that solving academic crosswords is a unique experience and it helps promoting generic learning skills.

Conclusion: A reasonably good response from final year pharmacy student in clinical pharmacy to crossword puzzle technique was observed. This model actively engaged the student in the learning process and represents an interesting way for student to learn course material generating enthusiasm and stimulating thought processes focused on the study of clinical Pharmacy.

O050-05 INFLUENCE OF ELECTRONIC TEACHING MATERIAL AVAILABILITY ON THE PHARMACOGNOSY STUDENTS SUCCESS RATE

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Introduction: Pharmacognosy is a subject that is taught in the second year of the Pharmacy Degree at the University of Murcia. Electronic teaching platform is a very important and useful tool for students, and is considered as a main factor of success for the new degrees associated to the Bologna plan.

Material and Methods: For the Pharmacognosy subject teaching, and specifically the General Pharmacognosy part, we established a plan of availability of electronic teaching material for the students. Half of the lectures of the General Pharmacognosy were imparted with full access to electronic material through the SUMA-Electronic-Platform (power point presentations, articles, etc; Part 1), and the other half was imparted without this supporting material, so that the students only had their hand-writing material taken by themselves (traditional notes) and recommended books (Part 2). Evaluation of the General Pharmacognosy was performed by a multiple choice test with 29 questions (15 for the group 1 and 14 for group 2). To test data homogeneity a Kolmogorov–Smirnov test was made. And a *t*-student test was performed to check for significant differences between both parts.

Results and Discussion: The Mean califications (\pm SEM) for part 1 was 7.28 ± 0.46 (over 10), And 6.74 ± 0.46 for part 2. The Kolmogorov test showed a normal distribution for both parts. Significant differences were found between part 1 and 2 after performing the *t*-student test ($P < 0.05$).

Conclusion: These results show that there are evident advantages for students learning through electronic supporting material, regard to the traditional notes method.

O051-05 PHARMACOLOGY IN THE NEWS

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Introduction: The group which is responsible for Pedagogical Innovation in the Teaching of Pharmacology (University of Valencia) has designed a new experience based on the follow-up of news in general and/or specialized press concerning pharmacology topics with two objectives: to develop the critical analysis of the scientific information that is transmitted in mass media, especially in written press, and to elaborate information about drugs addressed to either sanitary professionals or patients.

Methods: The topic selected was the suspension of the marketing authorisations for rosiglitazone in Europe but not in USA. This voluntary activity was addressed to students of 5th course of Pharmacy, who had to develop the following tasks: (i) to look for past information about rosiglitazone in newspapers; (ii) to look for scientific information and to

contrast it with the news reports; (iii) to answer a series of proposed questions and (iv) to write a report including their own evaluation of the information and their conclusion. Finally, once the reports are ready, the teacher collects them and hand them out randomly, asking the students to carry out an anonymous peer evaluation and to indicate the strong and weak points of the assigned report.

Results: The level of acceptance for this activity was very high: practically 100% of the students who attended the course participated in the activity and considered it as an adequate tool for improving the learning of Pharmacology.

Conclusion: The use of news reports proves to be an effective, holistic approach to motivate students of Pharmacology to critically analyze drug information.

O052-05 USE OF JOURNALISTIC AUDIO-VISUAL RESOURCES THAT FACILITATE THE 'LIFELONG LEARNING' AND THE ACQUISITION OF EXTRACURRICULAR SKILLS IN THE STUDIES OF PHARMACOLOGY AND PHARMACOTHERAPY

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Introduction: The aim of the project has been to turn a journalistic product into an educational resource that it allows 'the enjoyment of learning' and could facilitate the development of transversal competencies.

Material and Methods: The procedure consisted of an approximation to the technologies and principal characteristics of the work of the journalist of the diffusion of scientific and university information, inside an office of communication. In fact, basic directives were contributed on how spreading technical information, across different tools used in the journalistic daily task as the press release.

Results: The students carried out a series of tasks to perform in class, consistent in holders to elaborate and teaser text of an informative text for its diffusion in press for it one offered them didactic material: press releases, summaries of investigations, press kits of the Direction of Communication of the University of Seville and scientific journals. Also were instructed in the theory of the reversed pyramid. Likewise, an outreach video was projected.

Conclusion: The results show that this methodology constitutes a useful tool that increases the acquisition of transversal competencies allowing across the enjoyment of learning, knowledge and critical thought in students the pupils.

O053-05 MARK ASSESSMENT OF PHARMACOLOGY, PHARMACY AND THERAPEUTICS DURING THE LAST FIVE YEARS IN THE VETERINARY SCHOOL OF CORDOBA (SPAIN)

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Pharmacology, Pharmacy and Therapeutics is a subject learned in third year in the Veterinary School of University of Cordoba (Spain). Next year will be the last in which this subject will be offered in the traditional way. A new subject, corresponding to the new Degree in the Veterinary Studies will be starting. For this reason, we study the marks during the last 5 years in this subject to obtain data that will be compared with the Pharmacology and Pharmacy marks, the new subject for the new degree. We explain the format of the exam and how the student attendance (previously recorded) affects the global mark. Results show that 46.65% of students passed Pharmacology, Pharmacy and Therapeutics (with a

minimum value of 40.57% in the year 2009–2010 and a maximum of 51.09% in the year 2005–2006). The mean percentage of students who attended classes at least once is 79.19% (with a minimum value of 69.29% in the year 2005–2006 and a maximum of 92.77% in the year 2009–2010). The correlation between students that attended classes ver-

sus students that passed the exam was negative ($r = -0.7411$) and non-statistically significant. Nevertheless it shows a tendency that needs to be analyzed considering different factors such as mean attendance, exam format and others. However, had we not taken into account the student attendance factor, mark would have been worse.