PAIN AND INFLAMMATION

C001
THE VARIANT G-ALLELE OF THE HUMAN μ-OPIOID RECEPTOR GENE (OPRM1) IS RELATED TO HALLUCINATIONS DEVELOPMENT IN PATIENTS WITH CANCER AND NON-CANCER RELATED CHRONIC PAIN

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It has been reported that the human μ-opioid receptor gene (OPRM1) gene polymorphisms may alter opioid efficacy.
To determine efficacy and safety of treatment with opioids in relation to the polymorphism of OPRM1 gene in patients with cancer and non-cancer-related chronic pain.
An observational, retrospective non-interventionist study has been developed in patients between 18 and 90 years old with cancer and non-cancer related chronic pain (muscle-skeletal, neuropathic and visceral pain), with >6 months of evolution, EVA ≥5, which tolerated the opioid-treatment and had no cognitive impairment, followed at the outpatient pain management consult. Clinical history and detailed symptoms, intensity of pain, emotional state and alert state through the EVA, BIP, Euroq 5D and HADS tests and the OPRM1 gene polymorphisms were evaluated. The patients were assessed after 8 weeks and 3 months of follow-up.
190 patients, 67.2% women, mean age 57.7 ± 14.2 years old, basal EVA 6 ± 1.2 (4 minimum–9 maximum). The OPRM1 gene detected polymorphisms were: 60.3% AA allele frequency, 36.5% AG allele frequency, and 3.2% GG allele frequency. We observed (multivariate logistic regression) those patients with *G allele (AG and GG): (i) showed a lower intensity of their current pain (1/0.86), (ii) were treated fewer with Tapentadol, (iii) and had less needing for opioid rotation. Although they had a lower incidence of adverse reactions, they showed a significant increased incidence of hallucinations ($P < 0.05$).
The variant G-allele of the human μ-opioid receptor gene (OPRM1) was related to the development of hallucinations in patients with cancer and non-cancer chronic pain.

C010
A STAT6-DEPENDENT MACROPHAGE PHENOTYPE PROMOTES MUCOSAL REPAIR IN MURINE IBD BY ACTIVATION OF WNT SIGNALING

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M2 macrophages express Wnt ligands and play an essential role in mucosal healing, which constitutes a main goal in IBD-treatment. STAT6$^{-/-}$ mice had an impaired M2 polarization and a delayed wound healing. We analysed the role of STAT6 in Wnt ligands expression and the effects of M2 macrophages in wound healing in a murine model of colitis.
Peritoneal macrophages from WT and STAT6$^{-/-}$ mice were treated with LPS and IFN-γ (M1) or Il-4 (M2a) and the expression of Wnt ligands (qPCR) was analyzed. STAT6$^{-/-}$ mice received TNBS (3.5 mg/20 g mice, intrarectally) or vehicle (EtOH 40%) and 2 days later were injected with M2a macrophages (2×10^6 million, i.p.) obtained from WT (WT-M2a) or STAT6$^{-/-}$ mice (STAT6-M2a). Four days after TNBS, mice were weighted and after sacrificing, the colon length, mucosal histology and mucosal expression of Wnt target genes were determined.
RNA expression of Wnt2b, Wnt7b and Wnt10a was significantly increased in WT-M2a (4.0 ± 1.1, 2.8 ± 0.3 and 2.9 ± 0.5, respectively) and not in STAT6-M2a (0.9 ± 0.2, 0.9 ± 0.3 and 1.0 ± 0.1 respectively), compared with levels detected in non-polarized macrophages. The injection of WT-M2a vs. STAT6-M2a accelerated the recovery of body weight (98.1 ± 0.6% vs. 92.9 ± 1.1%, respectively), reduced mucosal damage (4.2 ± 0.9 vs. 6.6 ± 0.5) and diminished the expression of iNOS, TNFα and IL-1β (0.7 ± 0.1, 0.5 ± 0.1 and 0.6 ± 0.2, vs. 1.0 ± 0.1, 1.0 ± 0.1 and 1.1 ± 0.1, respectively). WT-M2a, and not STAT6-M2a increased mucosal expression of nuclear β-catenin (1.6 ± 0.1) and mRNA expression of c-myc (1.6 ± 0.2) and Lgr5 (2.1 ± 0.4).
A STAT6-dependent induction of Wnt ligands b y M2a macrophages activates mucosal Wnt signaling and accelerates wound healing in the TNBS induced colitis.

C015
MACROPHAGES DERIVED FROM HSPCS *IN VITRO* EXPOSED TO TLR AGONISTS SHOW A DIMINISHED ABILITY TO PRODUCE INFLAMMATORY CYTOKINES

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Toll-like receptor (TLR) agonists drive hematopoietic stem and progenitor cells (HSPCs) to proliferate and differentiate along the myeloid lineage *in vitro*, and direct TLR-mediated stimulation of HSPCs also promotes macrophage differentiation *in vivo* during infection. However, although TLR-derived cells exhibit myeloid characteristics, it is not clear whether they are functionally equivalent to macrophages derived in the absence of TLR activation. To deal with this issue we have determined the ability of M-CSF-derived macrophages from HSPCs, differentiated either in the presence or absence of pure TLR agonist (Pam3CSK4, a TLR2 ligand, and LPS, a TLR4 ligand), to produce inflammatory cytokines (TNF-a lpha and IL-6). HSPCs were exposed transiently (24 h) or continuously (7 days) to TLR agonists to evaluate the possible TLR-induce reprogramming of HSPCs. Results showed that: (i) continuous exposure caused increased macrophage yield in response to LPS and particularly to Pam3CSK4, whereas following transient exposure this increase was only observed in response to Pam3CSK4, and (ii) differentiated macrophages displayed a reduced ability to produce inflammatory cytokines (TNF-alpha and IL-6) upon subsequent stimulation with both TLR ligands. This lower cytokine production was observed following both transient and continuous exposure to Pam3CSK4 or LPS. These results indicate that macrophage function can be modulated by TLR-signaling in the HSPCs from which they are derived, and that this signaling causes a reduced ability to produce inflammatory cytokines. Therefore, this observation may impact the clinical utility of targeting TLRs on HSPCs in order to boost myelopoiesis and/or attenuate inflammatory responses.

C017
CIGARETTE SMOKE INDUCES FUNCTIONAL ARTERIAL CXCL16 EXPRESSION THROUGH NOX5 EXPRESSION AND RHOA/P38MAPK/NFKB ACTIVATION

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Cardiovascular disease (CVD) is a major co-morbidity in chronic obstructive pulmonary disease (COPD), however the mechanism by which it is developed are poorly understood. Chemokine synthesis and expression by endothelial cells is likely an important process underlying cell recruitment within the cardiovascular system in airway inflammatory diseases caused by cigarette smoke (CS). Therefore, the potential link between CXCL16 and CS-induced endothelial dysfunction were investigated.
Flow cytometry and immunofluorescence were used to determine CXCL16 expression on human umbilical artery endothelial cells (HUAEC). A flow chamber assay was employed to measure leukocyte arrest under dynamic conditions. Intravital microscopy was used to evaluate arteriolar leukocyte recruitment in animals exposed for 3 days to CS.
CS aqueous extract (CSE) at 1% induced CXCL16 expression on the arterial endothelium and its neutralization resulted in a significant inhibition of CSE-induced mononuclear leukocyte-HUAEC interactions (62%). CSE-induced CXCL16 expression was found to be dependent on Nox5 expression and subsequent RhoA/p38-MAPK/NFκB activation. *In vivo*, mice CS exposure for 3 days provoked a mild inflammatory response in the lung and enhanced CXCL16 expression in the cremasteric arterioles, resulting in leukocyte-arteriolar endothelial cell adhesion, which was significantly reduced in animals with a nonfunctional CXCR6 receptor.
CXCL16/CXCR6 axis blockade might be a new therapeutic target in the prevention and treatment of COPD-associated CVD.

C019
ACTIVATION OF AUTOPHAGY WITH TREHALOSE ACCELERATES WOUND HEALING IN STAT6$^{-/-}$ MICE

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A defective autophagy has been associated with a higher risk of Crohn's disease. We have demonstrated that STAT6 knockout mice exhibited an exacerbated colitis by TNBS. We aim to analyse the autophagic flux in STAT6$^{-/-}$ mice and to determine the role of activating autophagy in TNBS induced colitis.
A sample of colon and peritoneal macrophages were obtained from WT and STAT6$^{-/-}$ mice to determine the expression of autophagic protein markers (LC3II, p62) and PPAR-γ. On the other hand, STAT6 (−/−) received trehalose (3% in drinking water) and 3 weeks later were administered with TNBS (3.5 mg/mice, intrarrectally) or its vehicle (EtOH 40%). Mice were weighted daily and sacrificed 4 days after TNBS administration. Mucosal histology and levels of autophagic protein markers in the colon were analyzed.
Increased LC3II and p62 protein levels and decreased PPAR-γ were detected in both the intestinal mucosa and peritoneal macrophages obtained from STAT6$^{-/-}$ mice compared with those from WT mice, suggesting a reduction in autophagic flux. In STAT6$^{-/-}$ mice treated with TNBS, administration of trehalose vs. vehicle: (i) accelerated the recovery of body weight (97.4 ± 0.8% vs. 90.5 ± 3.4), (ii) decreased mucosal damage score (5.4 ± 0.4 vs. 7.4 ± 0.7) and (iii) decreased protein levels of p62 and increased LC3II.
STAT6$^{-/-}$ mice exhibited a blockade of the autophagic flux compared with WT mice. Activation of intestinal mucosal autophagy with trehalose improves wound healing and ameliorates TNBS induced colitis in STAT6 knockout mice.

C026
DIETARY HYDROXYTYROSOL ACETATE PREVENTS INFLAMMATORY RESPONSE AND JOINT DAMAGE IN MURINE EXPERIMENTAL ARTHRITIS

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Hydroxytyrosol acetate (HTy-Ac), a polyphenolic compound from extra virgin olive oil (EVOO) has exhibited anti-inflammatory and antioxidant activities among others. We investigated dietary HTy-Ac supplementation effect on collagen-induced arthritis (CIA) in DBA-1/J mice.
DBA-1/J mice were randomized in four experimental groups (10 animals per group): (i) Naïve group, (ii) Control group (CIA), (iii) HTy diet group (CIA-HTy) and (iv) HTy-AC diet group (CIA-HTy-Ac). After 6 weeks, arthritis was induced by type II collagen. Mice were sacrificed 42 days after first immunization. Blood was collected and paws were histological and biochemically processed.
HTy-Ac diet significantly reduced joint edema and cartilage destruction, preventing the arthritis development. Dietary HTy-Ac significantly decreased serum mouse and bovine IgG1 and IgG2a levels, cartilage olimeric matrix protein (COMP) and metalloproteinase-3 (MMP-3) levels, as well as, the pro-inflammatory cytokines levels (TNF-α, IFN-γ, IL-1β, IL-6 and IL-17A). Moreover, the activation of Janus kinase-signal transducer and activator of transcription (JAK/STAT), mitogen-activated protein kinases (MAPKs) and nuclear transcription factor-kappa B (NF-κB) pathways was drastically ameliorated. According to nuclear factor E2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1), the protein expressions were up-regulated in those mice fed with HTy-Ac.
HT y-Ac was able to improve the oxidative events and returned proinflammatory proteins expression to basal levels probably through JAK/STAT, MAPKs and NF-κB pathways. These results support the interest of natural diet component in the development of therapeutic products.

C027
INHIBITION OF INFLAMMATORY RESPONSES BY SOLIDAGENONE DERIVATIVES

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Toll like receptors (TLRs) exert essential functions in the activation of the innate immunity and are expressed in macrophages. Persistent TLR signaling triggered by exogenous or endogenous ligands plays detrimental roles in many inflammatory conditions. TLRs trigger specific intracellular signaling pathways in macrophages that culminate in the activation and nuclear translocation of NF-κB, which induces the expression of many genes involved in the production of inflammatory mediators. Diterpenes have been described as potential agents in the treatment of infection and inflammatory diseases. In this context, a series of labdane derivatives (S1–S12) were prepared from the diterpene solidagenone and their anti-in flammatory potential was evaluated on TLR-mediated inflammatory responses in macrophages.
Mouse peritoneal macrophages were cultured and activated with TLR-ligands: lipopolysaccharide (LPS), Polyinosinic-polycytidylicacid (poly I:C) and lipoteichoicacid (LTA) in the presence of diterpenes. Inhibi-

tory effects of the compounds on inflammatory mediators as nitric oxide (NO) and signaling pathways involved (NF-κB, MAPK) were analyzed by western blot.
From these compounds, solidagenone derivatives S6 and S8 showed the most potent anti-inflammatory effects due to the reduction of NOS-2 and COX-2 gene expression. Additionally, diterpene S6 modulated the TLRs-mediated inflammation responses induced by different ligands. The effects of S6 were not related to NF-κB inhibition, but MAPK signaling pathway was involved through inhibition of p-38 phosphorylation.
The anti-inflammatory effects of solidagenone derivatives are related to inhibition of MAPK signaling pathways in macrophages. Together with their low toxicity, these diterpenes could have potential therapeutic perspectives in inflammatory conditions.

C031
BEDSIDE-TO-BENCH EVALUATION OF PAIN: GRIP STRENGTH AS AN INDICATOR OF PAIN INDUCED BY INFLAMMATORY ARTHRITIS IN THE MOUSE

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Functional disability in patients with inflammatory arthritis negatively affects their quality of life. Grip strength deficits in these patients correlates to pain, but this parameter is poorly studied in rodents. Our goal was to study if grip strength could be used as a pain indicator in mice with inflammatory arthritis.
Joint inflammation was induced by periarticular administration of CFA (Complete Freund's Adjuvant) to the ankles of CD1 mice, and it was histologically characterized by hematoxylin-eosin staining. Grip strength was assessed using a grip-strength meter. We evaluated the effects on this outcome of the oral administration of analgesics f rom different pharmacological classes: opioids (oxycodone and tramadol), NSAIDs (ibuprofen and celecoxib) and acetaminophen.
Animals with inflammatory arthritis showed both a massive periarticular immune infiltration and a pronounced grip strength decrease. The maximum functional decline (≈50%) was produced at 1–3 days after the induction of inflammatory arthritis, and it was fully recovered after 21 days. Oxycodone and tramadol produced an almost complete recovery in the grip strength of arthritic animals, while ibuprofen, celecoxib, and acetaminophen produced a limited maximum effect (≈60%). These data are consistent with their analgesic efficacy in humans.
Joint inflammation induces a decrease of grip strength in mice. This measure of functional disability is sensitive to analgesic treatment, and it is therefore largely attributable to pain. Approaching the evaluation of pain from bedside to bench is expected to improve the translation of new analgesics from bench to bedside.

C037
COMBINED CHONDROITIN SULFATE AND GLUCOSAMINE IS MORE EFFICIENT THAN CELECOXIB IN REDUCING SERUM LEVELS OF COLL2-1, A CARTILAGE DEGRADATION BIOMARKER, IN PATIENTS WITH SEVERE OA

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The levels of Coll2-1, Coll2-1NO2 and Fib3-2 have been found to be elevated in serum of osteoarthritic patients and to vary with severity. The objective was to investigate soluble osteoarthritis (OA) biomarkers in knee OA patients treated with Chondroitin Sulfate (CS) + Glucosamine Hydrochloride (GH) or CElecoxib (CE) of the MOVES trial (Multicentre Osteoarthritis intervention trial with Sysadoa).
Coll2-1, Coll2-1NO2 and Fib3-2 were directly measured by immunoassays (ARTIALIS SA) in MOVES trial participants who gave their written consent for biomarker measurement and with at least one biomarker value at three time points (D0, D120 and D180). This population included 418 subjects, 215 receiving CS (400 mg) + GH (500 mg) 3 times/day and 203 receiving CE (200 mg/day) for 6 months.
Biomarkers values were not associated with age, sex, race, weight, height or BMI. There were no statistically significant differences between CE and CS + GH groups for any of the three biomarkers during the study. However, there was a trend in favor of CS + GH in reducing Coll2-1 at D180 ($P = 0.069$) and a trend in favor of CE to reduce Fib3-2 ($P = 0.055$). CS + GH induced a significantly greater decrease of Coll2-1 in the subgroups of patients with Kellgren & Lawrence grade III, with synovitis, in OMERACT-OARSI responders or in patients with WOMAC pain at baseline ≤369 compared to CE ($P < 0.05$) at D180.
CS + GH was more efficient than CE in reducing serum Coll2-1, particularly in a subgroup of patients with severe OA. This data indicates that CS + GH may down-regulate cartilage catabolism and are in accordance with the symptomatic benefits observed in clinical trials.

C038
EFFECT OF CHONDROITIN SULFATE ON SOLUBLE BIOMARKERS OF OSTEOARTHRITIS: HOW TO ANALYZE AND INTERPRET THE RESULTS FROM AN OPEN-LABEL TRIAL IN UNILATERAL KNEE OSTEOARTHRITIS PATIENTS

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The objective was to investigate the effects of chondroitin sulfate (CS) on the serum levels of biomarkers in patients with knee osteoarthritis (KOA).
Seventy-two patients with unilateral symptomatic KOA were involved in a post-authorization open-label study. Patients treated with CS (800 mg/day) were evaluated 5 times from D-30 to 6-month. Primary outcome was the % relative change in serum biomarkers (Coll2-1, Coll2-1NO2, Fib3-2). Secondary outcomes were pain evaluation (VAS) and function (Lequesne's Index). Responders and non-responders were classified according to OMERACT-OARSI recommendations. An original cut-off method was applied to categorize patients and interpret variations in serum levels of Coll2-1.
Patients showed no difference in the serum biomarkers levels at baseline. Most of the biomarkers levels decreased after 1 month of treatment but no significant differences were reported. However when considering responders and non-responders, a significant difference was found for Coll2-1 at 3 months ($P = 0.030$) and 6 months ($P = 0.038$). CS decreased pain and function incapacity throughout the visits ($P < 0.01$). Patients treated with CS were considered as metabolic responders according to an intra-batch cut-off of 21% for Coll2-1 (variation related to homeostasis). CS showed a drastic reduction (from 13% to 3%) of the proportion of patients with an increase of Coll2-1 > 21%.
CS was effective modulating the metabolic state of KOA patients through the reduction of Coll2-1 levels in responders and the disease's symptoms. This study also proposes a new approach for the analysis and the interpretation of the variation in biomarker levels and introduce the notion of metabolic responders.

C045
IN A 2-YEAR DOUBLE-BLIND RANDOMIZED CONTROLLED MULTICENTER STUDY, CHONDROITIN SULFATE WAS SIGNIFICANTLY SUPERIOR TO CELECOXIB AT REDUCING CARTILAGE LOSS WITH SIMILAR EFFICACY AT REDUCING DISEASE SYMPTOMS IN KNEE OSTEOARTHRITIS PATIENTS

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To compare the long-term effect of chondroitin sulfate (CS) vs. celecoxib (EC) on the symptoms and progression of knee osteoarthritis in a multicenter, randomized, double-blind controlled study.
Symptomatic knee osteoarthritis patients and synovitis were treated with CS 1200 mg/day or CE 200 mg/day for 24 months. Quantitative magnetic resonance imaging (qMRI) were performed at baseline, 12 and 24 months evaluating cartilage volume loss (CVL) and the synovial membrane, among others. Symptoms associated with osteoarthritis were also evaluated.
A total of 194 patients were included. CS treated patients had a reduction CVL at 24 months in the medial tibiofemoral compartment ($P = 0.013$) and global knee ($P = 0.054$) than CE. No difference between treatments in synovial thickness or bone marrow lesions. As well, both treatments elicited same reduction on symptoms with no difference between them: joint swelling/effusion 51% vs. 39% ($P = 0.246$); VAS pain reduction 49% vs. 55% ($P = 0.439$) and for WOMAC pain 46% and 53% ($P = 0.415$), improves functionality 42.6% vs. 44.5% ($P = 0.828$) in the CS and CE groups respectively at 24 months. Paracetamol consumption as rescue medication was similar (CS 584 vs. CE 472 mg/day) groups. The incidence of adverse events was similar in both treatment groups.
This trial demonstrated the superiority of CS over CE at reducing the progression of knee osteoarthritis structural changes with a similar symptomatic efficacy and the advantage of a better safety profile.

C046
COMBINED CHONDROITIN SULFATE AND GLUCOSAMINE VERSUS CELECOXIB FOR PAINFUL KNEE OSTEOARTHRITIS: *POST-HOC* ANALYSES BY KELLGREN AND LAWRENCE GRADE AND C-REACTIVE PROTEIN LEVEL FROM A RANDOMIZED, DOUBLE-BLIND, MULTICENTRE CLINICAL TRIAL

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The Multicentre Osteoarthritis interVEntion trial with Sysadoa (MOVES) was a phase 4, noninferiority, double-blind study comparing efficacy and safety of chondroitin sulfate (CS) plus glucosamine hydrochloride (GH) with that of celecoxib (CE) in 606 patients with knee osteoarthritis and severe knee pain. Patients were randomized to receive 400 mg of CS plus 500 mg of GH tid or 200 mg of CE qd. Both treatments elicited a 50% reduction in WOMAC pain at 6 months, without differences between them.
To further analyze the results obtained according to different patient subsets, we sought to compare, as a *post-hoc* analysis, the efficacy of CS + GH with that of CE on reducing severe pain (WOMAC pain > 300) according to Kellgren–Lawrence grade (2 or 3) and C-reactive protein (CRP) levels (≤3 vs. >3 mg/l) in the MOVES study population, to determine whether they presented different treatment responses.
In patients with Kellgren–Lawrence grade 3, there were no statistically significant differences between CS + GH ($n = 99$) and CE ($n = 96$) on WOMAC pain reduction at all time points, after 1, 2, 4 and 6 months. Equally, there were no statistically significant differences between CS + GH ($n = 85$) and CE ($n = 81$) in patients with CRP >3 mg/l at all time points. Consumption of rescue medication was the same in both treatment groups for Kellgren–Lawrence grade 3 and CRP >3 mg/l (P = ns for all between-group comparisons).
This *post-hoc* analyses indicate that CS + GH has equivalent efficacy to CE in reducing pain after 1 month in patients with painful knee osteoarthritis and Kellgren–Lawrence grade 3 or with CRP >3 mg/l.

C047
LONG-TERM EFFECTS OF TREATMENT ON THE PROGRESSION OF STRUCTURAL CHANGES IN KNEE OSTEOARTHRITIS: 6-YEAR FOLLOW-UP

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This study aimed to examine, for the first time, the long-term (6 year) protective effect of combined glucosamine (Glu) and chondroitin sulfate (CS) treatment on knee cartilage volume.
Participants from the Osteoarthritis Initiative (OAI) Progression and Incidence sub-cohorts who had MRI data on the target knee at baseline and at 6 years and with a JSW >1 mm, and information on the Glu/CS consumption were included ($n = 1593$). The participants were stratified into two main groups based on whether or not they had medial meniscal extrusion at baseline, and the former group ($n = 429$) were further stratified into subgroups based on their exposure time: not exposed, 1 year, 2–3 years, and 4–6 years. MRI assessments were done using fully automated quantitative technologies.
Findings indicate that in participants with knee osteoarthritis of mild-to-moderate severity, treatment with the combined Glu + CS significantly reduced the loss of cartilage volume in the global knee associated with the lateral compartment. Moreover, the extent of the treatment's positive effect was also found to be related to the exposure time to treatment, the protective effect at 6 years being significant in participants who were exposed to 2 or more years of treatment.
Findings indicate that in participants with knee osteoarthritis of mild-to-moderate severity, treatment with the combined Glu + CS significantly reduced the loss of cartilage volume in the global knee associated with the lateral compartment. Moreover, the extent of the treatment's positive effect was also found to be related to the exposure time to treatment, the protective effect at 6 years being significant in participants who were exposed to 2 or more years of treatment.

C058
ORAL EFFECTS OF METHOTREXATE IN PATIENTS WITH RHEUMATOID ARTHRITIS

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The methrotexate (MTX) is one of the most used anti-inflammatory medications for the rheumatoid arthritis treatment. It is associated to secondary effects on the tissue as the oral mucose. Different studies indicate that undesirable effects of this drug are frequent in the oral cavity with the presence of ulcers, gingivitis and other periodontal diseases. We have studied the oral condition of a group of patients which are taking MTX and comparing it at same time with a group of healthy individuals of similar age and gender.
The study is carried out at the Department of Reumathology of the Hospital Universitario La Ribera from December 2014 to its end with the corresponding consent. The patient are divided in two study groups: study group (patients taking orally or by injection MTX with dosage 10, 15, 20 and 25 mg during at least 24 months, and control group (healthy individuals). The parameters evaluated are oral hygiene level, mainly quantified by the evidence of plaque; the presence of dental decay, assessed by the CAOd index (teeth with caries/absent because of caries or permanent teeth with fillings); study of the periodontal condition of patients, analized by the probing depth by the periodontal index and the assessment of the insertion loss at the periodontal screening and recording; and assess and detect changes at the oral mucosity (tongue lateral edge and oral mucosa).
Statistical analysis was performed by database processing programme.
Our results indicate that there are no statistical differences between both groups related with age, gender or oral hygiene when compare the plaque, the bleed and periodontal screening and recording index.
Nowadays, we are getting the first results and we don't have final conclusions yet.

C059
GB12, AN ISOLATED MOLECULE FROM *PELTHOPHURUM DUBIUM*, DOWNREGULATES LPSINDUCED INFLAMMATORY RESPONSE THROUGH STAT3 INHIBITION IN CULTURED MURINE PERITONEAL MACROPHAGES

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Introduction: GB12, has been isolated from different parts of a number of plants. It exhibits antifungal and anti-inflammatory. Particularly, in the present study we investigated the *in vitro* antioxidant activity of GB12 from *P. dubium* as well as its antiinflammatory effects on lipopolysaccharide (LPS) activated murine peritoneal macrophages.
Material and methods: GB12 was isolated from the methanolic extract of bark of *P. dubium*, by column chromatography. The *in vitro* antioxidant activity was determined using DPPH and ABTS radical scavenging assays at concentrations of 0.1–3 mM. In this study, murine peritoneal macrophages were isolated and treated with GB12 in presence or absence of LPS (5 μg/ml) for 18 h. Cell viability was determined using sulforhodamine B assay and NO production was measured using the Griess reaction. Pro-inflammatory enzymes and transcription factors protein expression levels were detected by Western blotting.
Results: *In vitro* antioxidant assays revealed a superior reduction of DPPH radical by GB12 in comparison to Trolox antioxidant capacity (positive control) by more than 26%. Similarly, against ABTS radical the antioxidant capacity was exceeding 37% compared to Trolox. Without affecting cell viability, GB12 significantly reduced nitrites levels as well as the protein expression of inducible nitric oxide synthase, and STAT3 phosphorylation in murine macrophages exposed to LPS.
Conclusion: This study establishes that GB12 possesses *in vitro* antioxidant and anti-inflammatory activities on LPS-stimulated murine macrophages reducing pro-inflammatory mediators via Jak-STAT signalling pathway.

C061
EVALUATION OF THE LACTOBACILLUS FERMENTUM IN THE DCA EXPERIMENTAL MODEL OF IRRITABLE BOWEL SYNDROME: IMPACT ON ANXIETY BEHAVIOR

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At present, there is no ideal treatment for IBS that combines efficacy and safety. The use of alternative medicines is becoming attractive for many patients. In this study the effects of Lactobacillus fermentum was evaluated in a rat IBS experimental model induced by intracolonic administration of deoxycholic acid (DCA).
Male Sprague Dawley rats (240–320 g) were administered DCA once daily on 3 consecutive days, and received orally the probiotic (109 CFU per day) or Gabapentin (70 mg/kg). One and 2 weeks after, abdominal withdrawal reflex to colorectal distension (CRD) was semi-quantitatively scored. Also the referred pain was evaluated with Von Frey filaments. After 2 weeks, the open field test was performed to evaluate the stress associated to IBS in a novel environment. Then, all rats were sacrificed and the expression of disease markers were evaluated in the colonic tissue by qPCR: COX-2, the mucins MUC-2 and MUC-3, and the toll like receptor TLR-3.
The probiotic treated group showed lower CRD score values than IBS control, and also an amelioration of the referred pain. The open field test revealed that rats from IBS control group presented an altered behavior (decreased general activity) in comparison with normal rats, and the administration of the probiotic resulted in a restoration of the locomotor activity. The biochemical analysis of the colonic specimens revealed that Lactobacillus fermentum ameliorated the increased expression of COX-2, as well as that related with the toll like receptors TLR3. In addition, Lactobacillus fermentum was able to significantly counteract the reduced expression of the mucins, MUC-2 and MUC-3.
Lactobacillus fermentum exerts beneficial effects in this experimental model of IBS, together with an improvement of the stress associated with IBS. In addition, the probiotic was able to improve the altered immune response observed in IBS.

C063
INVOLVEMENT OF THE CHEMOKINE CCL3 AND CC CHEMOKINE RECEPTORS 1 (CCR1) IN INFLAMMATORY HYPERNOCICEPTION IN MICE

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Chemokines (chemoattractant cytokines) are widely known for their involvement in inflammation and, more recently, as modulators of nociception in pathological settings (Abbadie et al., *Brain Res Rev.* 60: 125–134, 2009). Besides the prominent role played by the CCL2/CCR2 axis in nociception, it has been reported that the chemokines CCL3 and CCL5, which bind to CCR1, are involved in nociceptive responses derived from cancer (Pevida et al., *Neuroscience* 259:

113–25, 2014) and neuropathic (Kiguchi et al., *Neurosci Lett* 484: 17–21, 2010) pain. Nevertheless, the CCR1 contribution to inflammatory nociceptive processing is poorly understood.
The aim of the present study was to investigate the role of CCR1 and its main ligands, CCL3 and CCL5, in the hypernociceptive responses in two standard models of acute (carrageenan) and chronic (complete Freund′s Adjuvant, CFA) inflammation in mice.
The systemic administration of the CCR1 antagonist, J113863 (3–30 mg/kg, 30 min.) dose-dependently counteracted thermal hyperalgesia measured by the unilateral hot plate test in both models of inflammation. Moreover, the administration of J113863 (30 mg/kg) also inhibited mechanical allodynia assessed by von Frey test in chronically inflamed mice. Neither paw size nor the presence of inflammatory cells was modified after the administration of the maximal antinociceptive dose of J113863. Besides, CCL3 mRNA and CCL3 protein expression measured by RT-PCR and ELISA respectively were dramatically increased in both acute and chronically inflamed paws, whereas CCL5 remained unaltered. Furthermore, no change in CCR1 paw expression was detected by western blot. The intraplantar administration of an anti-CCL3 antibody (0.3–3 μg) dose-dependently blocked thermal hyperalgesia in both inflammatory models, as well as mechanical allodynia (10 μg) derived from chronic inflammation. Altogether these data suggest that the peripheral increase of CCL3 seems responsible for hypernociception evoked through the stimulation of CCR1 in these particular inflammatory settings and that the blockade of CCR1 can be a useful strategy in the management of inflammatory pain.
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C064
CHARACTERIZATION OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS EXTRACELLULAR VESICLES AND THEIR EFFECT ON OSTEOARTHRITIC CELL PRIMARY CULTURES STIMULATED BY INTERLEUKIN-1β

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Extracellular vesicles (EV) are lipid bilayer particles excreted to the extracellular medium. Certain EV like exosomes (EX) and microvesicles (MV) participate in cell-to-cell communication by transfer of bioactive molecules. Adipose-tissue-derived mesenchymal stem cells (AD-MSC) release EV both under physiological and pathological conditions; the immunomodulatory properties of AD-MSC have proven to be beneficial in several diseases, such as osteoarthritis. We have investigated the effects of AD-MSC derived EX and MV in osteoarthritic chondrocytes (CO) and osteoblasts (OB) stimulated with interleukin-1β (IL-1β).
Cells were cultured with appropriate media supplemented with EV-free serum. EV were isolated from AD-MSC conditioned medium by differential centrifugation with size filtration. To characterize EV, transmission electron microscopy and resistive pulse sensing were used. CO and OB were stimulated with IL-1β (10 ng/ml) and treated with MV/EX for 24 h. Then, the inflammatory markers interleukin 6 (IL-6), tumour necrosis factor α (TNFα) and prostaglandin E2 (PGE2) were measured by ELISA and RIA.
MV and EX had a mean diameter of 316 nm and 115 nm, and a concentration of 8×10^9 and 3.8×10^{10} particles/ml, respectively. In CO, treatment with MV (3.6×10^7 particles/ml) and EX (7.2×10^7 particles/ml) resulted in a significant reduction of IL-6 but not TNFα nor PGE2 levels. In OB, the same treatment resulted in a significant reduction of PGE2 and IL6, but not TNFα.
Administration of EV may have potential pharmacological applications to lessen pathological inflammatory conditions. Differences in response between osteoarthritic cell types may lead to new and more specific therapeutic targets based on the interaction between AD-MSC derived EV and cells.

C065
EXTRACELLULAR VESICLES FROM FASCIOLA HEPATICA ADULTS ATTENUATES MUCOSAL INTESTINAL DAMAGE IN DEXTRAN SULFATE SODIUM INDUCED COLITIS IN MICE

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Inflammatory bowel diseases are a kind of recurring or chronic immune diseases of the gastrointestinal tract. Recently, supporting the 'hygiene hypothesis' many studies focuses on the immunomodulatory effects of extracellular vesicles (EV) from helminth parasites (1, 2).
The present study evaluates the protective and therapeutic effects of EV from F. hepatica adults on the inflammatory response in dextran sodium sulfate (DSS) induced colitis.
EV from F. hepatica adults obtained as previously described (3) were subcutaneously injected (10 μg/mouse, 10 μl) to mice once a week in three alternative weeks during 42 days before the induction of colitis. Then, ulcerative colitis was induced to C57BL/6 mice through oral administration of 3% DSS (w/v) in water for 7 days (4), starting at day 43. Mice were weighed during the experiment and their clinical status, including weight loss, stool consistency and/or diarrhea and presence of blood in stool was monitored. Animals were sacrificed by cervical dislocation at day 51, mice colon were harvested, weighted and the length measured and submitted to histological and biochemical analysis.
An absence of toxicity for immunized mice was observed. Pretreatment with EV from F. hepatica adults exhibited an amelioration of clinical symptoms as evidenced by macroscopic and histological findings. It improved disease activity index and protected from colon shortening (DSS mice 5.60 ± 0.06 cm vs. EV mice 6.60 ± 0.21, $P < 0.01$).
In conclusion, pretreatment with EV led to an amelioration of the inflammatory process and a protection of the intestinal mucosa in colitic mice.
1. Montaner et al., 2014. *Front Immunol* 5: 433.
2. Buck et al., 2014 *Nat Commun* 5: 5488.
3. Marin et al. 2013 *J Ethnopharmacol* 150: 925.
4. Marcilla et al., 2012 *PLoS One* 7(9): e45974.

C067
CHARACTERIZATION OF AN ANIMAL MODEL OF INFLAMMATION INDUCED BY CALCIUM PYROPHOSPHATE DIHYDRATE CRYSTALS

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A number of crystalline and particulate substances such as monosodium urate crystals (MSU) and calcium pyrophosphate dihydrate crystals (CPPD) provoke arthritis and are also associated with other destructive arthropathies. Both MSU and CPPD have been identified as agonists for the NLRP3 inflammasome. Numerous studies have demonstrated the ability of interleukin-1β (IL-1β) to induce cell infiltration and the expression of a wide range of inflammatory and

degradative mediators leading to articular destruction. The aim of this study was to characterize the inflammatory response induced by CPPD in the mouse air pouch.

To create the air pouch, sterile air was injected subcutaneously into the dorsal area of the mouse; 1 ml of saline or 1 ml of: CPPD (100 and 200 mg/pouch), MSU (100 and 200 mg/pouch), or lipopolysaccharide (LPS, 1 μg/pouch) in saline was injected into the air pouch. At different times (2, 6 or 16 h), mice were sacrificed and the exudate was collected. Cells present in exudates were measured with a Coulter counter and the levels of IL-1β and tumor necrosis factor-α were determined by ELISA. Caspase 1 activation in leukocytes was measured by a fluorogenic assay.

Injection of LPS or particulate substances resulted in a robust inflammatory response where CPPD induced the release of higher levels of IL-1β compared with LPS and MSU. Time-course studies revealed the presence of maximal inflammation 6 h after CPPD administration that may be related to caspase 1 activation. At this time point, administration of NaHS (300 μg/pouch) significantly down-regulated cell migration and the production of cytokines.

We have characterized a novel animal model of acute inflammation induced by CPPD that may be useful to target the inflammasome and novel therapeutic strategies in related conditions.

C068
ANALGESIC EFFECT EVOKED BY THE SPINAL ADMINISTRATION OF THE INHIBITOR OF DNAMETHYLTRANSFERASE 5-AZACYTIDINE IN CANCER-BEARING MICE. A PRELIMINARY STUDY

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Treatment of cancer pain is a challenge at clinical level since approximately 20% of patients suffering advanced cancer experience pain symptoms perceived as moderate/severe. Epigenetic is a rapidly advancing research field focussed on the regulation of gene expression through chromatin modifications mainly due to DNA methylations, histone acetylations or the influence of miRNA, without altering the primary DNA sequence. The possible role of epigenetic mechanisms in chronic pain processing has been suggested and, in particular, some studies performed in animal models of pain have shown that DNA methylation or histone deacetylation can be responsible for the development and maintenance of neuropathic and inflammatory pain. Therefore, the blockade of spinal DNA methyltransferase or histone deacetylases counteract neuropathic or inflammatory pain respectively (Wang et al., *Brain Res.* 1418: 64–69, 2011; Bai et al., *Mol Pain.* 6: 51, 2010). However, little is known about the epigenetic mechanisms that regulate bone cancer-induced pain. We have tested the influence of DNA methylation in a model of neoplastic pain developed in our laboratory induced by the intratibial inoculation of RM-1 prostate cells in immunocompetent C57BL/6 mice (Llorián-Salvador et al., *Prostate* 75: 70–83, 2015). The single peritumoral (300 nmol/100 μl) or intrathecal (30 nmol/5 μl) administration of the DNA methyltransferase inhibitor, 5-Azacytidine (5-AZA), 30 min before testing did not modify thermal withdrawal latencies or von Frey thresholds in tumor-bearing mice or evoke any apparent adverse reaction. Using a chronic schedule, the daily peritumoral administration for 3 consecutive days of 5-AZA (300 nmol) did not modify bone cancer-evoked hypernociception. In contrast, following a daily intrathecal administration for 3 days of 5-AZA (30 nmol) analgesic effects were observed in tumor-bearing mice from the 3rd day after administration. Our preliminary results suggest that the inhibition of DNA methylation can alleviate some hypernociceptive symptoms related to the presence of a painful tumoral process in mice.

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C069
THE SECRETOME OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS DECREASE CELL MIGRATION AND INFLAMMATORY MEDIATORS OF THE INNATE INFLAMMATORY RESPONSE

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Innate mechanisms of the inflammatory response are crucial for the resolution of pathological processes. Adipose tissue derived mesenchymal stem cells (AMSC) have shown protective effects against inflammation in part due to secreted factors (exovesicles). We have studied *in vivo* the paracrine effects of the AMSC secretome in the innate inflammatory response in mouse.

Zymosan-induced mouse air pouch model was used at 4 and 18 h to evaluate the effects of AMSC and their secretome (CM). Two types of exovesicles [microvesicles (MV) and exosomes (EX)] were determined in CM. Leukocyte migration and the level of inflammatory mediators were measured in exudates by ELISA or radioimmunoassay. Cyclooxygenase-2 and microsomal prostaglandin E synthase-1 (mPGES-1) expression was studied by western blotting and the phosphorylation of p65 nuclear factor-κB (NF-κB) by immunofluorescence. MV and EX concentration was 5.1×10^9 particles/ml and 2.8×10^{13} particles/ml, respectively in CM at Passage 1 of AMSC. All inflammatory parameters were significantly reduced by CM and AMSC at 4 h after zymosan injection with lower effects at 18 h. However, it is noteworthy that CM is more potent on the inhibition of leukocyte migration and myeloperoxidase production at 4 h than AMSC. Cellular migration reduction was associated with lower levels of chemokines and leukotriene B4, and down-regulation of mPGES-1 expression with the inhibition of prostaglandin E2 production. Moreover, Interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α and TNF-stimulated gene 6 levels decreased significantly. These anti-inflammatory effects are related, in part, to the inhibition of NF-κB activation.

The results support the potential utility of CM derived of AMSC as a new approach for early treatment of inflammatory diseases.

C071
EFFECTS OF RIOCIGUAT AND SILDENAFIL ON VENTILATION-PERFUSION COUPLING IN RAT LUNGS

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Current treatment for pulmonary hypertension (PH) with vasodilators is limited by their uncoupling effects on ventilation-perfusion due to the inhibition of hypoxic pulmonary vasoconstriction (HPV). We aimed to compare the effects of the new drug soluble guanylyl cyclase activator riociguat with the fosfodiesterase five inhibitor sildenafil on HPV in isolated rat pulmonary arteries (PA) and *in vivo* on ventilation-perfusion in rats.

HPV was recorded in isolated PA mounted in a wire myograph. Anesthetized (ketamine/xilacine) rats were catheterized to record PA pressure and exposed to hypoxia (10% O_2) or to unilateral bronchial obstruction. Ventilation and perfusion was analyzed by micro-CT-SPECT images in rats with pulmonary fibrosis induced by bleomycin.

Riociguat produced a more effective relaxant response in isolated PA than sildenafil. However, the potency of both drugs was similar under condition of high oxygen (95%) or hypoxia (0%). Both drugs inhibited

HPV *in vitro* and the vasoconstrictor response to U46619. *In vivo* the two drugs were more effective in inhibiting the increase in PA pressure induced by bilateral hypoxia or by U46619 than in inhibiting the unilateral hypoxia induced by bronchial obstruction. Pulmonary fibrosis was associated with ventilation-perfusion uncoupling. Both drugs increased lung perfusion but did not affect the ventilation-perfusion ratio.
Despite the inhibitory effect on HPV observed *in vitro*, the two drugs did not worsen ventilation perfusion coupling.

C073
INTRACELLULAR CALCIUM MEDIATES CELL CYCLE ARREST AFTER A2B ACTIVATION IN HUMAN KERATINOCYTES

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Adenosine, acting at its receptors (AR), regulates both inflammation and tissue repair of damaged skin. We have recently observed that activation of A2B AR, the most prominently expressed by epidermal cells, decreased keratinocyte proliferation (1). Therefore we studied the signal transduction pathways involved in this effect.
Normal human keratinocytes (NHK) isolated from foreskin of healthy young donors were incubated with adenosine (1 μM) or the selective A2B agonist BAY60–6583 (1 μM) in the presence or absence of selective A2B antagonist MRS-1706 (1 μM), the calcium chelator BAPTA-AM (10 μM) or the adenylyl cyclase activator forskolin (50 μM). After 24 h, cell cycle (BD Cycletes Plus DNA Reagent Kit) was determined by cytometry. Cell proliferation was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction at 48 h.
The G2/M fraction of untreated cells (19.32 ± 1.2) was significantly decreased after treatment with BAY60–6583 (14.7 ± 0.7, $P < 0.05$) and adenosine (13.0 ± 1.3, $P < 0.01$); whereas G0/G1 phase (66.9 ± 1.8 untreated) was increased by both AR agonists (BAY60–6583: 75.0 ± 1.3, $P < 0.05$; adenosine: 77.5 ± 1.2, $P < 0.01$). This anti-proliferative effect was reverted by the selective A2B antagonist as well as by BAPTA-AM, suggesting that calcium mediates, at least in part, the cell cycle arrest induced by A2B activation. In contrast, forskolin treatment significantly induced cell growth, indicating a proliferative role for cAMP signalling.
Although cAMP is a main player on A2B AR signalling, intracellular calcium and other signalling pathways can mediate the cell cycle arrest induced by A2B activation in NHK.
(1) Montesinos et al., (2014) Effect of adenosine receptors on keratinocyte function. *Purinergic Signalling*, 2014, DOI: 10.1007/s11302-014-9430-7, p17

C074
POTENTIAL TREATMENT OF IMMUNOBULLOUS DISEASES WITH A NOVEL PI3K δ INHIBITOR

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Immunobullous diseases such as pemphigus vulgaris and epidermolysis bullosa are characterized by the production of autoantibodies to structural components of the skin resulting in impaired keratinocyte adhesion and blistering. Finding ways to control autoantibody production may offer new therapeutic avenues for these diseases. PI3Kδ is mainly expressed in cells of the immune system and is essential to establish a proper humoral response. We have designed a novel potent PI3Kδ inhibitor (LAS191954) and assessed its pharmacological activity as an immune modulator.
LAS191954 therapeutic potential was assessed in cellular assays *in vitro* and in various experimental animal models of spontaneous autoimmunity and bullous disease.
LAS191954 inhibits BCR-mediated activation of human B cells *in vitro* and prevents primary and secondary antibody responses to KLH in immunized mice. In addition, LAS191954 dose-dependently decreases the production of autoantibodies to the pemphigus vulgaris autoantigen Dsg3 in MRL/lpr mice when administered daily for 4 weeks, with an efficacy similar to prednisolone. Furthermore, a single administration of LAS191954 dose-dependently prevents concanavalin A-induced T cell activation as measured by IL2 production in rats. Both B cell and T cell modulating activities *in vivo* are further enhanced in combination with corticosteroids, suggesting additive mechanisms of both drug classes. Finally and most importantly, LAS191954 alone normalizes clinical skin manifestations in a model of epidermolysis bullosa using a curative scheme.
LAS191954 shows immunomodulating properties and the ability to alter the course of immunobullous diseases.

C075
EFFICACY OF DEXAMETHASONE, TACROLIMUS AND TOFACITINIB TREATMENTS ON THE FITCINDUCED DELAYED-TYPE HYPERSENSITIVITY MODEL IN MICE

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Explore the impact of drugs with different mechanisms of action on the delayed-type hypersensitivity (DTH) model induced by FITC in mice. A corticosteroid (dexamethasone), a calcineurin inhibitor (tacrolimus) and a JAK inhibitor (tofacitinib) were tested using different treatment protocols. The induction was done on days 1–2 with FITC application to both ears, and on day 8 another FITC application was given to the right ear (challenge). Ear oedema and histological changes were analysed 24 h after challenge. Test compounds were administered orally (1–100 mg/kg) or topically (1–100 μg) during the sensitisationphase (days 1–5) or at the challenge (day 8).
Ear swelling was accompanied by a mixed dermal cellular infiltrate and epidermal pustules. Tested drugs showed differente efficacy profiles following their topical application. Tofacitinib (60 μg) demonstrated efficacy only when administered during the sensitisation phase (63% inhibition). Tacrolimus (20 μg) inhibited ear swelling in both regimens, but with a higher efficacy in sensitisation treatment than at challenge (96% vs. 46% inhibition). In contrast, dexamethasone (1 μg) suppressed ear oedema only at challenge (88% inhibition). By oral route tacrolimus and dexamethasone showed similar profile than by topical route, however high doses were needed for efficacy.
In the FITC-induced DTH model drugs are more efficacious by topical than by oral route, irrespective of their mechanism of action, suggesting that effector cells migrate to the skin during sensitisation and are activated in situ during challenge. The model is useful for the screening of novel topical drugs.

C076
CHLOROQUINE-INDUCED PRURITUS MODEL IN MICE, CHARACTERIZATION AND PHARMACOLOGICAL VALIDATION USING AN AUTOMATED PLATFORM

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Pruritus induced by the antimalarial drug chloroquine (CQ) has been used in mice to explore the mechanisms of action of limited drugs.

Scratching is usually evaluated by counting bouts from video-recorded animals. Main limitation of this method is the impossibility to distinguish real pruritus inhibition of the drug or a CNS side effect.
Our aim has been to characterize the CQ model and explore its utility for the screening of drugs using an automated recording system (Laboras, Metris) that in addition to scratching can measure spontaneous locomotion activity.
CQ subcutaneous injection produced the bell-shape scratching response previously reported. At the highest dose (32 mg/kg), lack of pruritus was accompanied by a decrease in spontaneous activity. No scratching was observed after oral CQ administration at 100 mg/kg. Quantification of CQ levels in different tissues (UPLC-MS/MS) suggested that skin levels were responsible for the pruritic effect. The pharmacological effect of TRPA1 antagonists was evaluated and nalfurafine was used as reference compound. Low doses of the TRPA1 antagonist A967079 were able to inhibit pruritus without major effects on locomotion activity.
The introduction of an automatic platform combining scratching and spontaneous locomotion activity will improve the understanding of the pathophysiology of pruritus as well as the site of action on antipruritic agents.

C077
IMPORTANCE OF THE NON-VERBAL COMMUNICATION (NVC) IN THE STUDENTS OF HEALTH SCIENCE

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The non-verbal communication is essential in the health professionals. A questionnaire has been passed to students of pharmacology and those of other areas of knowledge and educational levels to find out how do they value the NVC in different situations of their daily life. Those who took part were students of the University of Alicante (i) of Pharmacology and therapeutics of the nursery degree; (ii) use and abuse of medicines and drugs of the degree of criminology; from the University of Tucumán (Argentina): (i) odontology and (ii) Economic Science and from two secondary schools of Vocational Training, the IES Leonardo da Vinci of Alicante and the INS Gabriela Mistral of Sant Vicenç dels Horts in Barcelona. The results show three findings: (i) The students consider that the academic success also keeps relation with a good use of the NVC; (ii) there is no difference between students of sanitary degrees and those of other degrees; (iii) The university students value more the NVC that the ones of pre-university levels. Consdering all this we conclude that it is a transversal content to take into account in the curricular development.

C078
PHARMACOTERAPEUTIC MONITORING AND BLOOD PRESSURE CONTROL IN COMMUNITY PHARMACY

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Hypertension has a high prevalence in developed countries, and it is closely related to a high risk of morbi-mortality. Pharmacotherapeutic monitoring could be an essential tool, allowing the community pharmacist to control patient's disease. The objectives of this study were to evaluate the impact of blood pressure control in community pharmacy; to assess its influence on the degree of therapeutic adherence and changes in the patient's lifestyle; and to detect problems related to necessity, effectiveness and safety of medicines.
Two patients were selected at a community pharmacy. Following informed consent, the patients were interviewed. Their blood pressure was monitored using a Holter ambulatory monitor. The Morinski–Green test was used to evaluate the adherence to the treatments, and the modified Dader method to detect problems related with necessity, efficacy and security of those treatments.
We improved the adherence to the treatment in both patients. In one of them, a problem related with the necessity of the treatment was detected and solved, reducing the average blood pressure from 150/91 to 126/80. In the other patient, we detected and solved a problem related to the security and efficacy of the treatment, reducing the average blood pressure from 137/73 to 127/74.
In conclusion, the method employed for pharmacotherapeutic monitoring and blood pressure overseeing, allowed the control of hypertension and the avoidance of adverse effects of the drugs employed by the patients.

C109
DIVERGENT INFLAMMATORY RESPONSE TO POSTPRANDIAL TRIGLYCERIDE-RICH LIPOPROTEINS IN RETINAL-PIGMENTED EPITHELIUM CELLS

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Retinal pigment epithelium (RPE) plays a pivotal role in age-related macular degeneration (AMD), which is a progressive eye disease with no cure and limited therapeutic options. In the pathogenesis of AMD, degeneration of RPE cells by multiple factors including increased oxidative stress and chronic inflammation precedes the irreversible loss of photoreceptors and central vision. In the postprandial state, triglyceride-rich lipoproteins (TRLs) lead to a complex series of events potentially oxidative and inflammatory. The main goal of this study was to carefully characterize the effect of postprandial TRLs with different fatty acid composition in oxidative and inflammatory markers on RPE cells (ARPE-19). TRLs were isolated from serum of healthy volunteers after the ingestion of a single meal enriched in refined olive oil (monounsaturated fatty acids, MUFAs), cow's milk cream (saturated fatty acids, SFAs) or refined olive oil plus omega-3 long-chain fatty acids (MUFAs + ω-3). TRL-SFAs provoked the highest ROS generation and inflammatory response in ARPE-19 cells, followed by TRL-MUFAs and TRL-MUFAs + ω-3. Herein, we demonstrate for the first time that TRLs are metabolic entities able to induce RPE oxidative stress and inflammation in a fatty acid-dependent manner. Therefore, these exciting findings open new avenues for understanding the aetiology of AMD and provide opportunities for developing novel nutritional strategies with olive oil to prevent development and progression of geographic atrophy in AMD.

C111
TOPICAL APPLICATION OF GLYCOLIPIDS AND FUCOXANTHIN FROM MICROALGAE PREVENTSINFLAMMATION IN A MURINE MODEL OF TPA-INDUCED HYPERPLASIA

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Psoriasis is a chronic autoimmune skin disease characterized by inflammation in dermis and epidermis, keratinocyte hyperproliferation and infiltrating leukocytes that release cytokines. Microalgae have emerged as source of bioactive compounds, including glycolipids and carotenoids, with anti-inflammatory properties in different experimental models.
To evaluate the efficacy of a pharmaceutical formulation containing a glycolipid fraction (ISO42MD) or fucoxanthin isolated of Isochrysis galbana on a murine model of TPA-induced epidermal hyperplasia.
Permeability studies of cream, ointment and gel containing β-carotene were performed by using Franz diffusion cells and artificial membranes to determine the best formulation. Permeation of cream containing ISO42MD or fucoxanthin was also evaluated through rat skin. ISO42MD or fucoxanthin cream (100 mg, 200 μg/site) was applied to the shaved dorsal skin of female Swiss CD-1, 30 min prior to TPA administration (2 nmol/site), for 3 consecutive days. Histological analysis, cytokines production and inducible enzymes expression studies were performed.
The *in vitro* results showed the cream formulation presented the best permeability profile and the formulation with ISO42MD permeated more rapidly than fucoxanthin. Pretreatment with ISO42MD and fucoxanthin cream or the reference agent dexamethasone attenuated TPA-induced skin edema and improved histopathological features, with a reduction of inflammatory cell infiltrate. Both treatments inhibited proinflammatory cytokines release (TNF-α, IL-1β, IL-6, IL-17) as well as COX-2 and iNOS expression. Moreover, fucoxanthin significantly increased IL-10 levels and PPAR-γ expression.

C112
PRO-RESOLVING EFFECTS OF AN OXYLIPINS-CONTAINING BIOMASS FROM CHLAMYDOMONAS DEBARYANA IN A RECURRENT COLITIS MODEL IN MICE

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Microalgae are able to modulate their metabolism and produce bioactive molecules useful for the treatment of inflammatory diseases such as Inflammatory Bowel Disease (IBD). Microalgae are a source of $n-3$ PUFAs and derived-oxylipins, which have showed anti-inflammatory and anticancerogenic activities. Recently, our group has reported the preventive effects of an oxylipin-containing-lyophilized-biomass of the microalga Chlamydomonas debaryana (Cd) in an acute colitis model in rats.
To evaluate the efficacy of this lyophilized biomass of Cd on a trinitrobencenesulfonic acid (TNBS)-induced recurrent colitis model in mice.
The effect of lyophilized was studied on a moderate chronic inflammation model induced in female BALBc mice by repetitive intrarectal challenges with TNBS. Administration of lyophilized at different doses (L-50, L-100, L-200 mg/kg animal) started 2 weeks before colitis induction and continued throughout colitis development (38 day, triweekly).
The oral supply of lyophilized biomass of Cd caused a marked amelioration of weight loss and colon weight/length ratio, as well as reduced the immune cell infiltration, as was revealed by the histological study. Furthermore, the cytokine profile was improved with a reduction of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-17), and an increase of the anti-inflammatory cytokine IL-10. The lyophilized biomass also decreased the inflammatory enzymes expression (COX-2 and iNOS).
Our study confirms the anti-in flammatory properties of a lyophilized biomass rich in oxylipins and provide new evidence of its immunomodulating effects in colitis relapse models, similar to patients with IBD. The mechanisms involved may be related to the participation of pro-resolving molecules.

C125
PROLONGED ANALGESIA WITH MULTILAMELAR AND PEGYLATED UNILAMELAR LIPOSOMAL MORPHINE IN MICE

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Postoperative pain is generally treated with repeated opioid intramuscular injections, which is suboptimal, since it is difficult to sustain therapeutic effect with intermittent doses. This approach is also a problem for patients because of increasing risks of infection and respiratory depression. Liposomes are biocompatible phospholipid-based vesicles which may be used as a vehicle for drug delivery.
The aim of this study was to develop unilamelar (PEG-L) and multilamelar (MLV-L) liposomes for morphine drug delivery, to evaluate their physicochemical characteristics, and to compare pharmacokinetics and analgesia in mice after a single i.p. dose of free morphine or PEG-L/MLV-L.
MLV-L (HSPC:CHOL) were pre pared by thin film method whereas PEG-L (HSPC:CHOL:mPEG-DSPE) were prepared by high pressure extrusion method. Formulations were characterized for size distribution, drug entrapment and transmission electron (TEM). Pharmacokinetic was evaluated following morphine i.p. administration (30 mg/kg) using GC-MS. A standard hot-plate test was used to assess efficacy after 30 and 90 mg/kg dose.
Particle size was 120.45 ± 10.5 nm with an entrapment efficiency of 47.84% for PEG-L, and >5000 nm with an entrapment efficiency of 89.80% for MLV-L. TEM images showed a spherical shape for both of them. MLV-L showed the slowest morphine release rate and PEG-L even slower than free morphine. Duration of analgesia was significantly prolonged with the highest dose (90 mg/kg) of liposomal morphine (250 min for MLV-L and 380 min for PEG-L) compared to safe dose (30 mg/kg) of free morphine (150 min).
These results suggest that liposomal formulations may provide prolonged analgesia with single-dose administration.

C126
MITOCHONDRIA: A NEW CHONDROITIN SULPHATE THERAPEUTIC TARGET FOR OSTEOARTHRITIS

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We have demonstrated that mitochondrial dysregulation occurs in cartilage cells during osteoarthritis (OA). Here we investigated the mitochondrial activity of OA human articular chondrocytes treated with chondroitin sulphate (CS).
OA human chondrocytes were treated with CS (Bioibérica, Spain) with or without IL1β 5 ng/ml, TNFα 10 ng/ml and LPS 100 μg/ml.
A 64% increase in chondrocytes $\Delta\psi m$ was observed after 48 h treatment with CS 200 μg/ml. A significant depolarization was induced with TNFa. CS was also able to increase chondrocytes $\Delta\psi m$ up to 66%, partially counteracting the effect of TNFα. CS significantly increased ATP production compared to basal condition (0.55 μM vs. 0.65 μM). After TNFα stimulation, ATP production fell to 0.40 μM; CS was able to inhibit TNFα effect increasing the ATP synthesis up to

0.62 μM. CS was able to reduce NO synthesis induced by IL1β, TNFα or LPS. The NO levels were reduced after cytokine stimulation up to 21%, 32% and 31% respectively. In presence of IL1β, ROS production and SOD2 activity were increased. Intracellular ROS production as well as SOD activity decreased in CS treated chondrocytes at an average of 80%.
CS improves mitochondrial activity in human OA chondrocytes. The mitochondrial membrane hyperpolarization and the increased ATP production could correlate with a greater resistance of CS treated chondrocytes to apoptosis. Moreover, the reduction of NO and ROS levels, as well as the reduction in SOD2 activity, provide evidence of the effect of CS on oxidative stress regulation.

C129
ATTENTUATION OF HYALURONAN FRAGMENT INDUCED INFLAMMATORY RESPONSE IN MACROPHAGES BY CHONDROITIN SULPHATE

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Hyaluronan (HA) fragments (<500 kDa) are known to be able to induce an inflammatory response from macrophages. We have previously shown that chondroitin sulphate (CS) can attenuate the monosodium urate crystal mediated release of inflammatory cytokines from activated macrophages in culture.
Mature macrophages were primed with 10 ng/ml of LPS for 24 h with CS in various physiologically relevant concentrations. After 24 h, cells were added for 24 h more with various molecular weights (sizes; from 7.5 to 1.54 × 10^6 kDa) and concentrations of HA fragments.
HA fragments produced large increases ($P < 0.0001$) in IL-1β release at 10 and 100 μg/ml, with a decreasing gradient effect ($P < 0.0001$) seen from ULMW to MMW and no effect seen for HMW HA. CS (100–200 μg/ml) produced a dose dependent r eduction in IL-1β release in cells treated with 10 μg/ml of the 3 HA lower MW fragment types. While ULMW HA fragments induced a significant increase ($P < 0.0001$) in intracellular caspase-1 activity, CS had no effect on this activity. CS reduced intracellular IL-1β and proIL-1β in cells treated with ULMW HA, however, the ratio between the 2 was unchanged.
HA fragments (<289 kDa) induced an inflammatory response in THP-1 macrophages which could be attenuated by CS. An anti-inflammatory effect at the level of the inflammasome would be expected to decrease intracellular caspase-1 activity thereby decreasing the ratio of IL-1β to proIL-1β. Since we did not observe this, it can be concluded that the anti-inflammatory effect of CS is upstream of the inflammasome.

C136
URINARY BLADDER SIGMA-1 RECEPTORS: A NEW TARGET FOR CYSTITIS TREATMENT

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The sigma-1 receptors (S1-Rs) are involved in somatic pain but their implication in visceral pain is less explored and, in particular, their role in urinary diseases is still unknown. Therefore, we evaluate the presence of S1-R in urothelium and their implication in the cystitis induced by cyclophosphamide.
Human and mice bladder sections were used to locate the S1-R by western blot and immunohistochemistry. The implication of S1-R in cyclophosphamide cystitis was evaluated using wild-type (WT) and S1-R knockout (S1-KO) mice and selective S1-R antagonist (BD-1063, NE-100 and S1RA). Histopathological and biochemical damage of the bladders were measured by morphometric analysis, myeloperoxidase assay a nd western blot. The pain related behaviours were recorded for 4-h after cyclophosphamide injection and then the cutaneous referred hyperalgesia was measured by von Frey filaments.
S1-Rs are present in human and mice bladder urothelium. The histopathological (oedema, haemorrhage and desquamation), biochemical (myeloperoxidase activity and phosphorylation of extracellular regulated kinases 1/2 -pERK1/2-) and pain parameters indicatives of cyclophosphamide-induced cystitis were reduced in S1-KO mice in comparison to WT mice. The painful manifestations induced by cyclophosphamide were reversed by S1-R antagonists in WT animals, but not in S1-KO animals, indicating that the effect of the drugs was mediated by this receptor.
S1-Rs can modulate the urothelium injuries caused by cyclophosphamide and influence the biochemical parameters indicative of bladder damage, finally modifying the painful experience. Therefore, these receptors may represent a new drug-target for treatment of urinary bladder disorders.

C139
BMP7 DEFICIENCY CAUSES HYPERALGESIC PHENOTYPE AND REDUCED RESPONSE TO MORPHINE IN A MODEL OF NEUROPATHIC PAIN

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Transforming growth factors-β (TGF-β) constitutes a large family of multifunctional cytokines including, among others, TGF-βs and bone morphogenetic proteins (BMPs). We have previously reported that TGF-β1 prevents the development of allodynia after sciatic nerve injury. The objective of this study was to investigate whether a member of the BMP family, BMP7, is involved in neuropathic pain development.
Quantitative-PCR, western blot and 'in situ' hybridization were performed to determine the presence of BMP7 and its receptors in pain relevant areas. To induce neuropathic pain, mice were subjected to sciatic nerve injury and development of allodynia was assessed with von Frey monofilaments. To evaluate the antinociceptive effect of morphine in the chronic model of neuropathic pain, mice received cumulative doses of morphine (1–10 mg/kg).
BMP 7, ALK3 (BMPR1A) and ALK6 (BMPR1B) were expressed in pain-related areas within the Nervous System including cortex, spinal cord and dorsal root ganglion. BMP7 deficiency in $BMP7^{+/-}$ mice accelerated the development of neuropathic pain compared with wild-type mice. Four weeks after nerve injury, when both genotypes displayed equal degree of allodynia, mice received cumulative doses of morphine. $BMP7^{+/-}$ mice were significantly less sensitive to the anti-hyperalgesic effect of morphine at the doses of 3 and 6 mg/kg in comparison with wild-type mice.
BMP7 deficiency triggered a pro-allodynic response and reduced the analgesic response to opioid drugs in a model of chronic neuropathic pain. We suggest the potential value of BMP7 signalling as a therapeutic target for chronic pain. Supported by SAF2013-47434-R.

C140
DIABETES MODIFIES THE MECHANISMS INVOLVED IN THE RELAXANT ACTION OF ATRIAL NATRIURETIC PEPTIDE IN THE RABBIT BASILAR ARTERY

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The relation between diabetes and stroke is bidirectional: diabetes is an important risk factor for ischemic stroke and acute stroke frequently induces hyperglycemia. Moreover, plasma atrial natriuretic peptide (ANP) levels are raised in diabetes and stroke. We have studied how alloxan-induced diabetes might modify the effects of ANP in rabbit basilar arteries and the mechanisms involved in such actions.

Isometric tension in isolated rabbit basilar artery was recorded, prostanoids release and plasma ANP were measured by enzyme immunoassay.

Plasma ANP levels were higher in diabetic than in control rabbits. ANP induced endothelium-dependent relaxations of UTP-precontracted basilar arteries, with higher potency in diabetic than in control rabbits. NG-nitro-L-arginine inhibited ANP-induced relaxations, but this inhibition was lower in diabetic than in control rabbits. In control rabbits, indomethacin potentiated the relaxation to ANP. In diabetic rabbits, indomethacin enhanced the ANP-induced relaxation. In the presence of ANP the basilar artery released thromboxane A2 and prostacyclin, but the release of endothelial prostacyclin was decreased in diabetic rabbits. In KCl-depolarised arteries, relaxation to ANP was almost abolished both in control and diabetic rabbits. Iberiotoxin and 4-aminopyridin inhibited the relaxation to ANP in a similar way in both groups. However, the inhibition induced by glibenclamide was higher in diabetic than in control rabbits.

Diabetes enhanced the relaxant potency of ANP in the rabbit basilar artery; this enhancement could be related with an enhanced role of KATP channels and it would be partially counteracted by a decreased release of NO and endothelial prostacyclin.

C142
PERCUTANEOUS ABSORPTION OF TOPICAL ANTINFLAMMATORIES. A COMPARATIVE STUDY BETWEEN PREDICTED MODELS AND *IN VITRO* PERMEATION RESULTS

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Skin penetration of a topically administered drug depends on its physicochemical properties and also on the formulation. Herein we disclose the skin penetration profile of three structurally diverse anti-inflammatory drugs: clobetasol propionate, sodium diclofenac and tacrolimus. Additionally, a comparison of the profiles in Franz cell assay of the drugs dissolved in a common vehicle (propylene glycol, PG) vs. selected commercial formulations is also presented.

Theoretical skin permeability was predicted using the Mitragotri and Potts & Guy models. Skin PAMPA assay was used as a predictor of stratum corneum permeation in buffer solution. Franz cell experiments were conducted in pig skin to determine the amount of penetrated compound.

In theoretical models and Skin PAMPA studied drugs were ranged from the most permeable to the lowest as diclofenac > clobetasol > tacrolimus. In Franz cell assay good penetration profiles were obtained for the three drugs in PG, even for tacrolimus, where the enhancer effect of PG can be appreciated. Regarding the formulations, percutaneous absorption was decreased more than fourfold for diclofenac gel (Solaraze®) and clobetasol cream (Clovate®) compared to PG. However, when tacrolimus and clobetasol ointments (Protopic® and Declobán®) were tested, the same and even higher degree of penetration than in PG was found. It suggests an occlusive effect of the ointment promoting penetration.

Our results demonstrate that different skin penetration models are highly valuable despite their own limitations. A clear influence of vehicles and formulations has been observed, which helps to understand the exposure and efficacy of topical treatments.

TEACHING IN PHARMACOLOGY

C002
FILMED CLINICAL CASES IMPROVED COMMUNICATION SKILLS OF STUDENT OF PHARMACOLOGY IN PODIATRY DEGREE

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Solving clinical case by writing-report lacks of students sympathies and interest in many cases. Make a short film is a novelty that really like to the students and may improve their arguing and planning capacities and their communication skills.
To evaluate the impact of presenting and resolving clinical cases through filming a short-film in the communication skills development and in the learning of medicines in Podiatry undergraduate students.
A 2-year study in which students were invited to voluntarily form groups (3 students maximum). Each group has to design and film a short-film (8 min maximum) showing a clinical case in which medicines' use was needed to treat feet pathology. A camera, a mobile-phone's video editor or whatever they may use was allowed. The job of each group was supervised and helped by a teacher. The students were invited to present their work to the rest of the class. After each short-film projection the students were encouraged to ask questions if they wanted to do it. After all the projections the students voluntarily answered a satisfaction survey.
Students of Pharmacology of Podiatry Degree, n = 101, 55.6% female, 20 $\pm$ 1.3 years old were enrolled. 37 short-films showing a clinical case were made. The average time spent by students in making the film was 12.4 $\pm$ 8 h. The percentage of students which were satisfied with this way of presentation of the clinical cases was 75.2%.
Filmed clinical cases performed by student of Pharmacology of the Podiatry Degree improved their communication skills.

C003
MAY TELEVISION MEDICAL DRAMAS HELP IN THE TEACHING OF CLINICAL PHARMACOLOGY? A PRELIMINARY CONTENT ANALYSIS OF HOUSE MD

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Television medical dramas have become very popular among medical students. Some authors have suggested its interest as a teaching tool (1). House MD has been used to teach professional values and bioethics (2). However, data of its use to teach pharmacology have not been reported. The aim of the present study was to perform a content analysis of House MD to ascertain it may be useful in the teaching of clinical pharmacology.
We reviewed the first Spanish season of House MD (22 episodes). Several variables were analyzed in the plot: main topic, pharmacological issues and drugs used. Further, their value as a teaching aid in pharmacology was arbitrarily classified as A: high, B: moderate and C: low.
Many chapters had a central plot that refers partially (9, 41%) or completely (4, 18%) to clinical pharmacology. All of them except one considered pharmacological issues, mainly adverse drug reactions (18, 82%) or ethics of drug use (8, 36%); 118 drugs were used, mainly anti-infective (15, 14%), cardiovascular (13, 12%), CNS (12, 11%) and immunological and anticancer drugs (11, 10%). Seven (32%) chapters were scored as A, 8 (36%) as B and 7 (32%) as C when considering their interest for teaching.
House MD may be a rich source of pharmacological knowledge as the present content analysis shows. However, its usefulness, practicability and acceptability for teaching needs to be established by means of controlled studies in the target populations.
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(2) Weaver R, Wilson I. Australian medical student's perceptions of professionalism and ethics in medical television programs. *BMC Med Educ* 2011; 11:50.

C005
ALTERNATIVES TO THE USE OF ANIMALS IN HIGHER EDUCATION. VIDEOS AS A TOOL FOR LEARNING TO EVALUATE ANALGESIC ACTIVITY BY HOT PLATE TEST

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The aim of this project was to incorporate videos into practices of Pharmacology, as an alternative to the use of animals, to learn to assess the analgesic activity by the hot plate test.
Male mice (20 $\pm$ 2 g) were divided into three groups (n = 6) and the animals marked individually. Before drug administration, mice were habituated to a hot-plate apparatus maintained at 55 $\pm$ 0.1°C. The animals were placed on the hot plate and the time until jumping was recorded. After habituation each group received subcutaneously saline solution (control), morphine or drug problem. The jump time of each mouse was again evaluated at 10, 20, 30, 60 and 90 min after administration. The hot-plate assay was recorded on video in real time. Individual videos for each of the animals at every assay time were edited. The teacher instructed the students on the basis of the practice and its performance. The students visualized the movies for each animal and the jump times were measured at every time. The results were plotted as mean $\pm$ SEM, analyzed and discussed during practical performance. After that, the students' opinion about this semi-virtual essay was requested.
All the students were involved and considered crucial the support from the teacher. Most of them fully agree with this alternative to the laboratory practices.
This project was supported by the USAL (ID2014/0171). The authors thank Digital Production and Innovation Service (USAL) for recording and editing videos.

C020
SEMINARS WITH PATIENTS AND HEALTH PROFESSIONALS: AN EXPERIENCE TO LEARN PHARMACOLOGY

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The Faculty of Health Sciences integrates the degrees of Pharmacy, Medicine, Dentistry, Nursing and Physiotherapy. Pharmacology is a compulsory subject in all our degrees, so it is an ideal subject for enhancing the student's multidisciplinary teamwork and facilitates the acquisition of knowledge and skills required by the various health professions.
Our Faculty offers seminars for all students in the various degrees, under a debate-discussion format with patients and health professionals who bring their experience and a multidisciplinary approach to pharmacological treatment.
The activity is set within the school calendar for all our degrees and students are offered the possibility to obtain one European Credit Transfer System (ECTS) for attending a minimum of seven seminars and submit a report for each seminar attended.
During the last 3 academic years we have offered a total of 23 seminars. They have been attended by a total of 397 students; 31 of them aimed to obtain ECTS. Attendance was variable ranging from 14 to 69 students. Most students (70%) attending these seminars were enrolled in our Pharmacy and Physiotherapy degrees. Student satisfaction was analyzed; 97.1% of students graded this activity with a degree of satisfaction of at least 70%. The best rated seminars were those on diabetes and mental health.
This activity improves comprehensive and multidisciplinary training amongst pharmacology students and allows them to obtain some of the skills required to work in a health team.

C022
PHARMACOLOGY CORE COMPETENCES REQUIREMENTS IN MAIN SPANISH BACHELOR DEGREE PROGRAMMES

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To set teaching objectives, it is essential to define the core competences for a subject area. This study aims to analyse the current competences in pharmacology for obtaining bachelor's degrees in Spain.
We identified 17 major areas of study at Spanish universities including subjects related to pharmacology. We selected the five in which pharmacology had the greatest bearing: biology, pharmacy, medicine, odontology, and veterinary science. We analysed the white books (reference guidelines for each area of study published by the Spanish government) for these five to extract the competences in pharmacology. Afterwards, we randomly selected 25% of Spanish universities for each area of study and compiled the pharmacology competences outlined on their websites.
We observed vast heterogeneity in the white books' specifications of the competences in pharmacology as well as in the criteria, definitions, and contents used to classify them. A total of 130 bachelor degree programmes (from the five previously selected areas of study) in 30 Spanish universities contained pharmacology courses. Information about specific competences provided on websites was very heterogeneous and not always sufficiently detailed. The competences varied widely among programmes, even among courses taught at the same university.
We recommend that the pharmacology core competences should be reviewed and updated in both the white books and university websites. We also suggest a common structure and content to be defined for drafting white books. University websites should provide transparent information ab out degree programmes and about competences in pharmacology, and this information should be provided in a uniform way.

C028
STANDARDIZATION OF CATALAN-LANGUAGE DRUG NAMES IN THE FRAMEWORK OF THE NEW ENCYCLOPAEDIC DICTIONARY OF MEDICINE

Guardiola Buxeda M.

Fund. Dr. Antoni Esteve

The Diccionari Enciclopèdic de Medicina (Encyclopaedic Dictionary of Medicine; DEM), a medical dictionary in Catalan, was first published in 1990. DEM contains more than 70,000 entries in 40 subject areas, one of which is pharmacology. To ensure that DEM keeps up with the constant advances in science and medicine, the 'Encyclopaedic dictionary of medicine in Catalan project' (DEMCAT) was established in 2009. Our role in this project is to review and update all the terms corresponding to drug names.
The Centre for Terminology in the Catalan Language (TERMCAT) undertook the data migration from the original dictionary, DEM, to its own terminology system, GdT (terminology manager). To standardize the terminology, we reviewed and updated the 4496 entries in the pharmacology subject area, using available terminology resources as references.
We selected around 2000 entries corresponding to drug names, reformulating and improving the definitions, synonyms, acronyms, equivalents in other languages (Spanish, English, and French), and complementary notes in these entries. We devised a terminology template for drugs to facilitate the inclusion of the information required in a definition: type of drug, action, uses, and route of administration. This template will be used to review all drug-name entries from DEM. Establishing clear guidelines for entries will improve the quality and structural homogeneity of the current and future entries, benefiting both terminologists and scientific professionals.

C040
DIRECT TO PATIENT ADVERTISING OF PRESCRIPTION DRUGS: OPINION SURVEY AMONG PATIENTS AND DOCTORS

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Direct-to-patient information (DTPI) by pharmaceutical companies is a topic under debate along Europe. The aim of this study is to find out significant differences between patients and prescribers about pharmaceutical DTPI on drugs subject to medical prescription.
A cross-sectional and descriptive study, conducted through anonymous and face-to-face surveys, to patients and primary and specialized care physicians from Valencia was carried out. The main variable was the level of agreement with DTPI of prescription drugs collected by a linear analogue scale, where five was established as the cut off value between agree/disagree. Sociodemographic variables and answers to short questions related to DTPI, influence on consumption and price of medicines were registered. Doctor-patient relationships were also recorded. Results were expressed as means with standard desviations (SD) and confidence intervals (CI) or alternatively, in absolute values and percentages.
From 09/2014 to 02/2015 surveys were conducted to 415 physicians (48.6% male; 46.2 (SD 12.4), CI 95%:45.0–47.4 years) and 363

patients (48.5% male; 46.5 (SD 16.0), CI 95%: 44.8–48.1 years). Statistical analysis revealed that both groups disagree with the DTPI: average scores of 3.03 (SD 14), CI 95%: 2.64–3.42 for physicians and 3.95 (SD 3.28), CI 95%: 3.61–4.29 for patients were obtained. Doctors [68% (281)] and patients [53% (193)] agree that DTPI would increase the price of prescription drugs. The vast majority of physicians (92%) and patients (73%) consider that consumption would increase. Most doctors (56%), but not patients (37%), believe that DTPI wouldn′t modify doctor-patient relationship.
Both groups disagree with DTPI and believe that it would increase both consumption and price of medicines, being these statements more evident in case of physicians.

C051
POSTER PRESENTATION AS AN EFFICIENT GROUP ACTIVITY IN LEARNING PHARMACOLOGY

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The work presented here is part of the 'Innovation in Teaching' project of the Medical Chemistry and Pharmacology Educational Motivation Group (QUIMIFAR; a GIE 'Grupo de Innovación Educativa') at the Universitat Jaume I in Castellón, Spain. General Pharmacology (3rd year; 7 credits) is one of the core subjects of the Medicine Degree taught at this university. It covers all major drug classes and their relevant features such as mechanisms of action and clinical applications. In order to familiarize the students with these concepts, as part of the practical classes of this subject, we designed a group activity in which they presented posters (2–4 students/poster; A1 paper size) describing a specific drug. A total of 28 posters were exhibited (14 posters/group) in two working sessions (3 h each) designed to resemble a mini-congress. Each poster contained the most important information regarding the drug in question, under the following headings: 1. General knowledge, 2. Pharmacokinetics, 3. Pharmacodynamics, 4. Clinical use, Indications, administration routes and treatments, 5. Drug interactions, 6. Adverse reactions, 7. Bibliography.

C053
PHYSIOTHERAPY STUDENTS FEEDBACK ON PHARMACOLOGY

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Pharmacology is an ever-changing subject whose knowledge is continuously updating. Feedback from students could serve as an effective tool to assess how the subject is developing in class. The objective of this study was to get feedback from second-year physiotherapy students enrolled on Pharmacology.
Pharmacology is an ever-changing subject whose knowledge is continuously updating. Feedback from students could serve as an effective tool to assess how the subject is developing in class. The objective of this study was to get feedback from second-year physiotherapy students enrolled on Pharmacology.
A total of 28 students (75% female and 25% male) participated voluntarily and returned the questionnaire (63.6%). Some of them (25%) had previous knowledge on Pharmacology before entering the second course. 57.2% agreed that Pharmacology of analgesia and inflammation is the most interesting topic for Physiotherapy students, followed by cardiovascular Pharmacology (17.8%) and general concepts on this subject (10.7%). They considered that teaching methodologies used made easier to understand concepts. Most of them (93%) mentioned that they studied Pharmacology only by teachers' class notes to prepare for their exams. 57.1% recognised that the primary motivation to study the subject was to pass their examinations, and only 17.8% have studied regularly to gain more knowledge. Most of them believe that the best evaluation systems are multi-choice questions (50%) or a combination of multi-choice questions and short answers (46.4%). For them, Pharmacology shows an intermediate level of difficulty, mainly due to the wide range of contents (89.3%). Finally, they found this subject useful and interesting for physical therapists (60.7%) or useful, interesting and important from a practical point of view (28.6%).
Feedback surveys provide important additional information on subjects taken by students. From our results we can conclude that our students from the Degree in Physiotherapy have positive attitudes towards Pharmacology, although some approaches should be explored to improve motivation to study regularly.

C055
A PROPOSAL FOR SKILL EVALUATION IN A PRACTICE OF VETERINARY PHARMACOLOGY

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Educational proposals made by the European Higher Education Area (EHEA) require new evaluation approaches. Scoring rubrics are considered an innovation tool to collect evidences of skill acquisition, as they provide a good approach of the students' work in a wide range of subjects. Thus, the aim of this study was to implement a competence evaluation method by rubrics as a way for documenting skill improvement in a practice developed in the subject Veterinary Pharmacology.
An outcome-based assessment rubric containing several performance indicators was specifically designed to evaluate abilities related to update their knowledge, synthesize and communicate information, read and interpret scientific data in English, and work in team.
A total of 132 students (74.2% females and 28.8% males) have been evaluated according to this method. Students acquired all the skills defined for the practice, reaching 86.6 $\pm$ 9.7% of those competencies previously defined. Female students demonstrated to have acquired a significantly higher level of skills (87.3%) than male students (85.0%; Mann–Whitney *U*-test, $P < 0.05$). The highest score was reached for the competences related to teamworking and the ability to interpret information in English, whereas the lowest values corresponded to that competence related to their capacity to keep knowledge updated and to synthesize information.
Standardized rubrics become helpful tools for the teaching staff to assess competencies, and to improve the outcomes of the learning process, although it is necessary the active involvement of all the teachers who collaborate in the subject.

C057
INTERDISCIPLINARY COLLABORATION IN THE PRACTICAL TEACHING OF PHARMACOLOGY: USE OF EXPERIMENTAL ANIMALS AND APPLIED ROBOTICS

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The use of experimental animals in practical teaching of pharmacology in Spain has dropped considerably over the past two decades and has been replaced by alternative proposals, mainly using computer programs. This methodological change came undoubtedly marked by European Directives published regarding the protection of animals

used for experimental and other scientific purposes (Directive 86609/EEC, Directive 2010/63/EU).
A practical lesson for Veterinary, Nursing and Biotechnology students has been designed to be held in two sessions. In the first session, the first three chapters of the Directive 2010/63/EU and its transposition into RD 53/2010 on animals used in experimentation ... including teaching, will be analyzed.
In the second part, students will evaluate the action of a neuroleptic, promazine, using mice, a maze and RCX Mindstorms robots programmed to navigate the maze by students of Computer Architecture.
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In the second part, students will evaluate the action of a neuroleptic, promazine, using mice, a maze and RCX Mindstorms robots programmed to navigate the maze by students of Computer Architecture.
The development of this practice demonstrated the importance of interdisciplinary collaboration in teaching, as well as the great degree of involvement of students of different courses when shared a practical class.

C060
DESIGN SERVICE-LEARNING PROJECTS IN THE PHARMACY DEGREE OF THE UNIVERSITY OF VALENCIA

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In the service-learning methodology, students provide service in their community that is directly connected to their academic coursework, and the community provides an educational experience for the student. The intention is to develop teaching skills with strategies that will lead our students to be sensitive to the social problems of their environment and experience citizen participation linked to their future professional and community service.
Teams of 4–5 students from the Clinical Pharmacy and Pharmaceutical Care course, a subject of the 5th year, as a part of the seminar sessions. The students, supervised by the teacher, selected the context of the service and defined both the service and the learning objectives, stressing how important it was that paid attention to the real needs of their environment. In seminar sessions, each group of students presented the designed project, and answered to the questions that may arise. The evaluation of each project was performed both by students and by teachers.
A total of 190 students participated. They presented about 38 projects, generally related to problems of drugs and nutrition in elderly or in young pregnant women at risk of exclusion. 83% of students thought that the development of the project would reinforce the learnings of Pharmaceutical Care.
This activity was very attractive to students because they can develop related training such as the rational use of medicines and health education activities.

C062
INNOVATION IN DRUG TRAINING WITHIN NURSING DEGREE AT UNIVERSITAT ROVIRA I VIRGILI (URV)

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A 'medication error' is commonly defined as any preventable event that may cause or lead to inappropriate medication use or patient harm while the medication is in the control of the health care professional, patient, or consumer. Such events may be related to professional practice, health care products, procedures, and systems, including compounding, dispensing, distribution, administration, education, monitoring, and use. Nurses spend 40% of their working time on tasks related to medication. Therefore, it is important to ensure comprehensive drug training within nursing degree.
The objective was to measure the efficacy of a continuous evaluation model based on competences.
Pharmacology curriculum is divided into the four Miller stages: (i) 'know' through lectures, (ii) 'know how' through discussions (topics not developed before), (iii) 'show how' through workshops (information sources, administration and dispensing) and (iv) 'do' by a clinical case (includes all of the above). We evaluated 231 URV students of nursing degree during 2014–2015.
Results are shown as mean score/% presentiality (maximum 100 points) for each Miller stage: (i) 'Lectures': 53.3/97.4; (ii) 'Discussions' 76.6/89.1; (iii) 'Workshops' 78/92.2; (iv) 'Clinical case': 79.4/93.5. Overall, 81.6% of the students obtained a score above 50 in Pharmacology.
The mean score of students improves throughout the different Miller stages of competences acquired ($53.3 < 76.6 < 78 < 79.4$). 'Discussions' have less presentiality, maybe because they need oral communication skills (11% not attending). Almost all students conduct the 'Clinical case', which is the highest stage, and, as said, scores are the highest compared to the other stages.
In conclusion, a continuous evaluation model based on competences may be effective. However, almost 20% of students take fewer than 50 points in total, so we need to improve the teaching-learning methodology to optimize not only the efficacy but also the efficiency of the curriculum.

C088
THE CASE METHOD TEACHING IN PHARMACOTHERAPY

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The case method is a teaching approach that uses decision-forcing cases to put students in the role of profesionals faced with decissions. It is based on the active and cooperative participation of students and the dialogue in real pharmacotherapeutic situations. This method was developed for students of five degree course of Pharmacy in the subject of Pharmacotherapy during the course 2014–2015. From the approach of the case method, the students should be able to comprehend, understand and analyze a given pharmacotherapeutic context and the variables involved in it.
The objectives of the case method were:

- To compare the real case presented with a theoretical model, identifying the peculiarities of the case and its ambiguities.
- To work with a professional approach, analizing a real problem, with its elements of confusion, sometimes contradictories, as occurs in reality.
- To propose strategies for solving the case, implementing and evaluating the results.
- To develop interpersonal skills, self-directed learning and team work.

The method developed consists of two phases:

1. Individual work: each student selected a patient from his personal or professional environment, conducted an interview with this patient and analyzed their situation.
2. Teamwork (5–6 students). The results of the interviews were analyzed. Some cases were selected by consensus of the team, in basis of their interest to the general discussion.

The evaluation was performed using headings, known in advance by students. It was developed in three levels: self-assessment, peer assessment and teacher assessment.

C093
HOW TO GAMIFICATE A HEALTH SCIENCE LESSON

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Gamification consists on the implementation of game-based techniques into a non-game based context and represents an interesting strategy when flipping a classroom. With the aim to encourage active participation of our students in classroom, we introduced gamification in the subjects of Pharmacology of the degrees of Pharmacy (Universitat de Valencia), Dentistry and Physiotherapy (Universidad Europea de Valencia) and Immunology of the degrees of Pharmacy, Medicine and Veterinary (Universidad Cardenal Herrera-CEU).
Throughout the academic course 2014–2015 we have used three different applications: Kahoot, a cell phone based application; the Space Race (Socrative), in which students compete in a virtual race; and the Pearson Quiz Show, that simulates a gamble play. All of these are versatile games that can be used both individual or collectively, in order to review previous knowledge on a topic or to reinforce the knowledge gained during the lesson.
Student motivation during the activity was remarkable, so it was the competitiveness established between them. Furthermore, some of these strategies allow the students the possibility to evaluate the activity, rating items such as entertainment, satisfaction and learning outcomes. This led us to make a real estimation of the impact of such type of activities, which has been highly satisfactory (95% of satisfaction).
Gamification in the context of Pharmacology and Immunology in different fields of knowledge represents a very interesting method to promote the interest of our students in our subjects, while contributing to gain some of the general competences included in our degrees.

C095
TRANSVERSAL EDUCATION IN THE DEGREE OF NURSING: IMPRESSIONS ABOUT THE INTEGRATION OF THE SUBJECT OF PHARMACOLOGY IN A CLINICAL SIMULATION ACTIVITY

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In the environment of the EHEA and the Bologna Declaration, clinical simmulation represents a very interesting and innovative education strategy. In the degree of Nursing, simulation is mainly used in practical subjects such as Nursing Care or Clinical Internship.
With the aim to reinforce the theoretical frame of the subject of Pharmacology, and to help our students to acquire a global and practical vision of what they are studying, we decided to integrate Pharmacology into a simulation activity, together with Physiopathology and Nursing Care for Health Altereations I, which belong to the second course of our Degree of Nursing.
This actitivy took place in the simulation classroom, using the simulation model ASL Simulator (Laerdal), where we set up a series of cases in which the use of drugs had a central role, so that the students could go further into aspects such as drug security, medication errors or medical order interpretations, which are all indispensable when applying reliable and high-quality Nursing Cares (Libro Blanco de Enfermería, ANECA).
From this experience we deduce that clinical simulation allows the integration of transversal and theoretical subjects such as Pharmacology, and this can increase the students motivation within the subject. From the teachers perspective, this activity represents a very interesting resource to create an interdisciplinar and collaborative environment that can be very useful for our students. It would be interesting to go further and extent these sessions to other degrees, setting out a blended simulation between students from different Health Science Degrees.

C105
DIFFERENCES IN FINAL SCORE IN THE DEGREE OF PHARMACY ACCORDING TO MCT EXAM OR MCT AND SHORT QUESTIONS EXAM

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Universidad de Murcia

In the last years use of multiple choice test (MCT) by University teachers has become the preferred method to evaluate the students. It has been also applied to several degrees like Law or those of Health Sciences which traditionally it has been important to memorize and write about long topics that could be corrected in a subjective way. MCT method is unbiased and facilitate correction but for other people sometimes do not provide real perception of knowledge and it is more difficult to obtain great results. For these reasons, the aim of this study was to compare the final score of Toxicology between students examined only with MCT and students examined with MCT plus short questions.
Exam 1 was composed by 50 MCT questions and exam 2 had 40 MCT and twelve short questions. Each MCT question had four possible answers and four mistake penalized one correct answer. Both types of exam had the same duration, 90 min.
Final score for exam 1 was 5.13 meanwhile final score for exam 2 was 5.95. T-student test comparison showed statistically significant differences between both types of exam ($P < 0.05$) being worse the marks obtained when the whole exam is based on MCT questions.
We highly recommend to include questions different to MCT questions in order to evaluate more properly knowledge acquired in a concrete subject. Sometimes MCT questions are not well understood or produce interpretation mistakes. We suggest that combination of both methods offer more information, better score and more accurate results.

C107
COOPERATIVE WORK OF MULTIDISCIPLINARY TEAMS OF STUDENTS (NURSING, PHARMACY AND MEDICINE) IN A SIMULATION CLASSROOM OF CLINICAL SKILLS

Garrigues Teresa M.[1], *Casal Angulo M.C.*[2], *Mitsuf L.*[3], *Garrigues Gil V.*[4], *Ferriols-Lisart R.*[5], *D'Ocon M.P.*[1], *Ferrándiz M.L.*[1], *Garrigues T.M.*[1], *Fernández-Garrido J.*[6]

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Four core competencies for health professionals have been identified to work efficiently in multidisciplinary teams: values/ethics, role/responsibility, communication and teamwork. To work these skills, a new teaching experience has been developed. The project was based on the methodology of resolution of cases through the cooperative work of a multidisciplinary team of students. A total of 51 students in the final year of Nursing (4th year; $n = 14$), Medicine (6th year; $n = 16$) and Pharmacy (5th year; $n = 21$) participated. A team of two students of each degree was constituted randomly and faced a clinical case in an advanced simulation classroom of clinical competence. The team performance was observed by the other students, as they answered a rubric on technical and nontechnical aspects. After the simulation, a debriefing was held among the participating students and teachers involved in the project. All participants discussed the reactions of the constituted team and reached a consensus on what would have

been the ideal performance to attain the best result for the patient care. We emphasized nontechnical skills as teamwork, effective communication and leadership as learning objectives. The simulation process and the subsequent discussion were filmed so the experience can be reviewed and used with other groups of students. The assessment of this learning-teaching method was carried out by means of a review of the rubrics and a satisfaction survey conducted at the end of the session. Both evidences support a high interest of this activity.

C118
THE USE OF BLOGS AS AN INNOVATIVE TEACHING METHOD FOR PHARMACOLOGY AND PHARMACOTHERAPY III AND CLINICAL PHARMACY SUBJECT

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The use of the blog promotes the collaborative learning, the exchange of ideas, among students and teachers, and skills development in analysis and critique.

To evaluate the use of blog as an interactive teaching tool and compare it with the crossword puzzles for learning of Pharmacology and Pharmacotherapy III and Clinical Pharmacy subject, taught at the fifth year of Pharmacy Degree at the University of Seville.

Blog was created with WordPress®. For crossword activity, students were divided into 10 working groups, which created 10 definitions. Once reviewed by teachers, puzzles containing 20 definitions were made with Eclipse Crossword® and solved in classroom. To assess student satisfaction, they were asked to complete an anonymous survey.

We observed a significant increase in the number of visits and visitors to the blog, reaching the maximum peak before the final exam. As regards crosswords, students were evaluated according to the number of right answers. The winning team was the one that solved the game more quickly. Unsolved crosswords were discussed in classroom and solutions were uploaded to the blog. The survey results showed that a high percentage of students considered the use of both blog and crossword enhanced participation and facilitated learning. The newest methodology for the students was the blog. Most of them positively assessed these innovative methods and recommended its use for next years, especially the blog.

The use of both blog and crosswords is an effective approach to motivate students and improve the learning of Pharmacology.

CARDIOVASCULAR PHARMACOLOGY

C004
RICE BRAN ENZYMATIC EXTRACT PREVENTS ENDOTHELIAL DYSFUNCTION AND RESTORES STRUCTURAL, MECHANICAL AND MYOGENIC ALTERATIONS OF RESISTANCE ARTERIES IN APOE$^{-/-}$ MICE

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Small mesenteric artery resistance and functionality are key factors for maintaining blood homeostasis. Structural, mechanic and myogenic alterations and endothelial dysfunction undergo upon diverse pathologic conditions leading to aggravation of cardiovascular diseases. We aimed to evaluate rice bran enzymatic extract (RBEE) effects in the structural, mechanic and myogenic properties and endothelial dysfunction present in ApoE$^{-/-}$ mice.

Seven week-old ApoE$^{-/-}$ mice were fed high f at diet (HFD) supplemented or not with 1 or 5% RBEE (w/w) for 23 weeks. Small mesenteric arteries were mounted in a pressure myograph and structural, mechanical and myogenic properties were calculated from pressure-diameter curves. Vascular reactivity was assessed by the ability of acetylcholine to induce relaxation in presence or absence of different combinations of inhibitors (L-NAME, indometacin, apamin and charybdotoxin). eNOS, P-eNOSThr495, P-eNOSSer1177, IKCa and SKCa expression were measured by Western Blot. Dihydroethidium fluorescence and elastin autofluorescence were evaluated by confocal laser scanning and collagen content was assessed by picrosirius-red staining.

ApoE$^{-/-}$ mice showed structural and mechanical alterations, alleviated by RBEE supplementation. 1% RBEE supplementation reduced superoxide production while 5% RBEE supplementation decreased collagen content. EDHF was the main responsible for C57Bl/6J endothelium-dependant relaxation showing increased expression of IKCa and SKCa, while NO release was more relevant for ApoE$^{-/-}$ mice. HFD reduced NO released due to inhibitory Thr495 phosphorylation in of eNOS, which was fully counteracted by RBEE supplementation.

ApoE$^{-/-}$ mice showed alter mechanical, myogenic and functional parameters which were softened by RBEE diet supplementation.

C007
SHORT-TERM PROTECTIVE EFFECTS OF DEHYDROHISPANOLONE ON MYOCARDIAL INFARCTION IN RATS

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Novel interventions directed to efficiently protect the heart from myocardial ischemia/reperfusion (I/R) injury are needed. In this context, a therapeutic approach is the search for cardioprotective agents. We have previously described that labdane diterpenes exert cardiac protective effects through activation of specific survival signals and inhibition of death pathways in cardiomyocytes. In this study, we investigated the short-term (3 days) protective effects of the diterpene dehydrohispanolone on post-ischemic recovery and the mechanisms involved.

Myocardial infarction (MI) was induced in Wistar rats by occlusion of the left anterior descending coronary artery, for 30 min. Diterpene (5 mg/kg) was intravenously administered concomitantly with reperfusion. Infarct size was used as endpoint. Histological analyses were performed.

A remarkable cardioprotection on I/R injury in rats was observed when the diterpene was administered early in the reperfusion, as evidenced by reduction of infarct size of heart (31.4 $\pm$ 3.1% vs. 57.1 $\pm$ 2.1%) at 3 days-postinfarction. This was accompanied by a significant decrease of myocardial interstitial fibrosis in diterpene-treated rats at 3 and 21 days, indicating that this compound prevented myocardial remodeling. Activation of AKT-dependent mechanisms and inhibition of apoptotic death also contributed to cardioprotection in the rat MI model.

The study demonstrated that the diterpene dehydrohispanolone confers *in vivo* early cardioprotection. Therefore, diterpene s may provide new potential therapeutic strategies to control cardiac reperfusion injury leading to improved clinical outcomes.

C008
INCREASED CELLULAR EXCITABILITY, IONIC ALTERATIONS AND ITS CROSS-TALK WITH HYPERTENSION DEVELOPMENT BY CHRONIC ETHANOL CONSUMPTION IN CHROMAFFIN CELLS OF NORMOTENSIVE AND HYPERTENSIVE RATS

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Chronic ethanol (EtOH) consumption and enhanced activity of the sympatho-adrenal axis have been associated with the development of hypertension. However, the sequence of alterations in the cellular homeostasis and electrophysiological properties from adrenal chromaffin cells (CCs) remain poorly understood. Therefore, this study aimed to establish the correlation between chronic EtOH consumption and alterations in ion currents. Male normotensive Wistar Kyoto rats (WKYs) and Spontaneously Hypertensive (SHRs) were subjected to the intake of increasing EtOH concentrations in their drinking water (5–20%). EtOH caused increase in systolic blood pressure (SBP) and cardiac hypertrophy in both rats, as well as massive generation of H_2O_2 and enhanced activity of ALDH. We found in CCs from WKYs and SHRs rats chronically treated with EtOH the following differences in the electrophysiological properties: (i) diminution of ICa accompanied by increase in the time of inactivation of Cav; (ii) augment of the cytosolic calcium concentrations in CCs from WKYs; (iii) decrease of IK(Ca) and IK(V) carried through K^+ voltage-dependent channels caused by EtOH metabolism products; (iv) increase of resting membrane potential in CCs from WKYs, and augment in spontaneous action potentials. All the results together, suggest that chronic EtOH consumption leads to an increase in activity of the sympatho-adrenal axis that may contribute to increase in SBP and aggravates hypertension in SHRs. This possibly occurs due to the ability of EtOH metabolism products inhibit mostly K^+ outward currents, thus triggering a cell depolarization and increased excitability which may be involved in the pathogenesis of hypertension.

C016
STUDY OF THE ANTI-INFLAMMATORY ACTIVITY OF EXENATIDE ON TNF α-STIMULATED ARTERIAL ENDOTHELIUM

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Exenatide is a glucagon-like peptide 1 (GLP-1) analog that is used in the treatment of type 2 diabetes (T2D). However, recent observations suggest that in addition to its antidiabetic action it can exert pleiotropic cardiovascular protective effects, improving endothelial dysfunction and preventing atherosclerosis. Therefore, in the present study we have investigated the anti-inflammatory activity of exenatide on TNFα-induced arterial endothelial dysfunction.
Parallel-plate flow chamber assay was employed to evaluate the effect of exenatide on mononuclear leukocyte adhesion to TNFα (20 ng/ml)-stimulated hum an umbilical artery endothelial cells (HUAEC). Flow cytometry and immunofluorescence was used to determine cell adhesion molecule and chemokine expression on HUAEC and ELISA to quantify chemokine release.
Human mononuclear arrest to TNFα-stimulated HUAEC was concentration-dependently inhibited by exenatide pretreatment through GLP-1R interaction. Exenatide 100 nM reduced TNFα-induced Intercellular Adhesion Molecule-1 (ICAM-1), Vascular Cell adhesion Molecule-1 (VCAM-1) and fractalkine (CX3CL1) endothelial expression. In parallel, the GLP-1 analog was also able to diminish the release of CXCL8/IL-8 and CCL2/MCP-1 promoted by TNFα stimulation of endothelial cells.
These results indicate that exenatide exerts protective cardiovascular effects through the inhibition of mononuclear cell adhesion to the arterial endothelium, a key event in atherogenesis. Reduction in TNFα-induced mononuclear cell recruitment was found to be through downregulation of endothelial cell adhesion molecule expression and inhibition of chemokine generation and release.

C024
VITAMIN D3 ATTENUATES ANGIOTENSIN-II INDUCED ABDOMINAL AORTIC ANEURYSM IN APOE$^{-/-}$ MICE AND ANGIOGENESIS.

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Abdominal aortic aneurysm (AAA) is a vascular disorder characterized by chronic inflammation of the aortic wall. In humans, low levels of Vitamin D3 have been associated with AAA development; however the potential direct effect of Vitamin D3 on AAA remains unclear.
Objective: To examine the effect of the oral treatment with calcitriol on AAA progression. Methods: Calcitriol treatment was evaluated in an experimental model of AAA induced by Angiotensin-II in apoE$^{-/-}$ mice. Incidence and diameter of AAA was determined. Leukocyte infiltration, angiogenesis (CD31^{+}), matrix metalloproteinase-2 (MMP)-2, MMP-9 and tissue inhibitor of metalloproteinase (TIMP)-1 were measured in the aorta by immunohistochemistry and RT-PCR.
Calcitriol treatment reduced the incidence of AAA and the expansion of the suprarenal aorta. The Vitamin D receptor (VDR) agonist reduced inflammatory infiltration (macrophages and neutrophils) and neovessel formation (CD31^{+}) re sulting in a significant decreased of chemokines (CCL2, CCL5 and CXCL1) and VEGF expression in aneurysm tissues. The ameliorative effects of calcitriol on Ang-II-induced aneurysm were also associated with the inhibition of elastin degradation, downregulation MMP-2 and MMP-9 levels along with increased expression TIMP-1.
Calcitriol attenuates Angiotensin-II-induced AAA formation by regulating inflammatory responses and extracellular matrix homeostasis suggesting a novel strategy for the treatment of AAA.
This study was supported by grants CPII13/00025, PI012/01271 and SAF2011-23777, from the Carlos III Health Institute, the Spanish Ministry of Health, the Spanish Ministry of Economy and Competiveness, and the European Regional Development Fund (FEDER).

C025
CALCITRIOL INHIBITS ANGIOTENSIN-II INDUCED LEUKOCYTE-ENDOTHELIAL CELL ADHESION AND CHEMOKINE RELEASE IN HUMAN UMBILICAL ENDOTHELIAL CELLS

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The migration of leukocytes into inflamed tissues involves a cascade of molecular events regulated by chemokines. VDR receptors, a member of the nuclear receptor superfamily, forms heterodimers with several nuclear receptors including RXR and mediate many biological effects. Objetive: To characterize the functional role of VDR agonism in vascular inflammation induced by Angiotensin-II.
Human umbilical vein endothelial cells (HUVECs) were used for these studies. HUVECs were stimulated with Angiotensin-II (Ang-II) and some groups were incubated with the VDR agonist calcitriol (1–100 nM) 20 h prior to Ang-II stimulation. Mononuclear leukocyte-endothelial cell recruitment was determined under dynamic flow conditions by a Flow chamber assay. CCL2, CCL5 and CXCL1 levels were determined by ELISA. In some experiments, cells were transfected with a RXRα specific siRNA to knockdown RXRα expression.
In human endothelial cells, calcitriol inhibited Ang-II induced mononuclear cell recruitment and CCL2, CCL5 and CXCL1 production in a concentration dependent manner. However, in cells transfected with RXRα siRNA, the inhibitory effect was abrogated. Immunoprecipitation and immunofluorescence analysis revealed that calcitriol effects were mediated by increased VDR-RXR^5-interactions.
Treatment with calcitriol may constitute a new alternative and effective therapy in the control of the vascular inflammation associated to cardiometabolic disorders.
This study was supported by grants CPII13/00025, PI012/01271 and SAF2011-23777, from the Carlos III Health Institute, the Spanish Ministry of Health, the Spanish Ministry of Economy and Competiveness, and the European Regional Development Fund (FEDER).

C032
EPICUTANEOUS APPLICATION OF TOLL-LIKE RECEPTOR 7 AGONISTS IMIQUIMOD LEADS TO ENDOTHELIAL DYSFUNCTION AND HYPERTENSION IN MICE

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The aim of this study was to evaluate the development of hypertension and endothelial dysfunction on new model of inducible Systemic Lupus Erythematosus (SLE) in wild-type (WT) mice by Toll-like receptors (TLR)-7 activation through epicutaneous application of TLR-7 agonist Imiquimod.
Ten-weeks-old female BALB/c mice were randomly divided into two groups: Control (Ctrl) and SLE. Mice from SLE group were treated on the right ears topically, 3 times weekly, with 1.25 mg of 5% Imiqui-

mod cream (Mochida Pharmaceutical) for 8 weeks. Systolic blood pressure (SBP) and heart rate were measured by tail-cuff plethysmography and by intraarterial register. At the end of the experiment, serum autoantibody and proteinuria levels as well as endothelial function were evaluated. Vascular NADPH oxidase activity and immunological abnormalities were analyzed by lucigenin-enhanced chemiluminescence assay and quantitative reverse transcription–polymerase chain reaction, respectively.
After 8 weeks of Imiquimod treatment, mice developed systemic autoimmune disease by increasing 4.5 times the plasma level of anti-dsDNA, and proteinuria. Imiquimod also induced a progressive raise in SBP (approx. 20 mmHg) and impaired endothelial-dependent acetylcholine relaxation, which was reversed by apocynin Furthermore, vascular reactive oxygen species (ROS) level, NADPH oxidase activity, and mRNA levels of both NADPH oxidase subunits NOX2 and p47phox and inflammatory cytokine IL-6 were increased in Imiquimod treated mice.
Imiquimod treatment leads to hypertension and endothelial dysfunction. The present study provides a novel protocol to study cardiovascular complications in a model of inducible SLE in wild-type mice.
Work supported by Junta de Andalucía (P12-CTS-2722) and RIC (RD12/0042/0011 and RD12/0042/0016).

C035
LACTOBACILLUS FERMENTUM CECT5716 CONSUMPTION IMPROVES HYPERTENSION AND ENDOTHELIAL DYSFUNCTION INDUCED BY TACROLIMUS: ROLE OF T CELLS

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Tacrolimus may cause endothelial dysfunction and hypertension by reducing regulatory T cells and increasing T-helper 17 cell polarization and inflammation. Lactobacillus fermentum CECT5716 (LC40) modulate the human immune.
The aim of this study was to analyse the effects of LC40 in the changes on blood pressure and endothelial function induced by tacrolimus in mice, focusing on the involvement of T cells.
Tacrolimus (1 mg/kg/day, ip, for 7 days) increases systolic blood pressure, which was partially prevented by L C40 (5×10^8 CFU/day). Endothelial-dependent relaxation to acetylcholine was also impaired by tacrolimus. Incubation with tempol abolished the impairment in acetylcholine relaxation. Tacrolimus also increased aortic DHE staining, and the mRNA levels of NOX1 and NOX4. In addition, tacrolimus increased the vascular phosphorylation of IκBα and the mRNA levels of TNFα and IL-6, but reduced mRNA levels of IL-10 and the protein expression of $FOXP3^+$. Tacrolimus also reduced Treg cells and increased Th17 cells. LC40 treatment prevented the impaired endothelium-dependent vasodilator responses to acetylcholine, the increased vascular NADPH oxidase-driven superoxide generation and mRNA levels of TNFα, and IL-6 and NFκB activation, increasing IL-10 and $FOXP3^+$ expression. LC40 restored the imbalance between Th17/Treg induced by tacrolimus.
LC40 prevented the raise on SBP and improved endothelial dysfunction induced by tacrolimus. These effects seem to be associated with reduction of vascular oxidative stress and vascular inflammation as a result of a decreased Th17 and increased Treg cells polarization.
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C039
MECHANISMS INVOLVED IN THE NEUROPROTECTIVE EFFECTS OF THE SELECTIVE ESTROGEN RECEPTOR MODULATOR, BAZEDOXIFENE, IN ACUTE ISCHEMIC STROKE

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Introduction/Objectives: The selective estrogen receptor modulator, bazedoxifene (BZA), reduces ischemic brain damage. The present study aims to gain insight into the molecular mechanisms involved in such a beneficial effect by investigating: (i) expression of estrogen receptors (ER) α, β and GPER; (ii) caspase-3 activity; and (iii) regulation of PI3K/Akt and MAPK/ERK1/2 signaling pathways. For comparative purposes, the study was run in parallel with 17β-estradiol (E2).
Material/Methods: Male Wistar rats subject to transient middle cerebral artery occlusion (tMCAO), were distributed in the following treated groups: vehicle, E2 (plasma concentration 45.6 ± 7.8 pg/ml) and BZA (plasma concentration 20.7 ± 2.1 ng/ml). At 24 h from the onset of tMCAO, RT-PCR, Western blot and histochemical analysis were performed on all brain samples.
Results: Ischemia/reperfusion reduced ERα and ERβ expression while it did not change the expression of GPER. BZA increased ERα and ERβ expression without changing that of GPER, while E2 did not change ERβ and GPER expression but did increase the expression of ERα. Both BZA and E2 inhibited the ischemia/reperfusion-induced increases in both caspase-3 activity and ERK1/2 levels. By contrast, neither BZA nor E2 changed the ischemia/reperfusion-induced increases in Akt levels.
Conclusions: These results show that its action on both ERα and ERβ as well as the subsequent modulation of the ERK1/2 signaling pathway accounts, at least in part, for the inhibitory effect of BZA on the stroke-induced apoptotic cell death. These findings suggest that BZA could be a potential neuroprotective drug in stroke treatment.
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C048
DOES ASTAXANTHIN HAVE SUPEROXIDE ANION SCAVENGING ACTION?

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Astaxanthin has demonstrated antioxidant activity *in vitro* and *in vivo*: (i) Superoxide anion ($^{\cdot}O_2^-$) generated by pyrogallol autooxidation elicits endothelial dysfunction and (ii) Our objective was to evaluate the ability of astaxanthin to provide vasoprotection focused in superoxide scavenger properties.
Methods: Concentration-response curves to acetylcholine (ACh, 10^{-8}–10^{-4} M) and sodium nitroprusside (SNP, 10^{-9}–10^{-5} M) were performed in aorta of Wistar rats. Responses were studied after incubation with pyrogallol (10^{-5} M). The effect of astaxanthin (10^{-6} and 10^{-5} M) and SOD (100 UI/ml) on impairment of relaxation induced by pyrogallol was evaluated.
The $^{\cdot}O_2^-$ levels were measured by lucigenin-enhanced chemiluminescence, assessing those generated by pyrogallol in Krebs solution or produced by stimulation of vascular NADPH-oxidase with β-NADH

(10^{-4} M). $^{\cdot}O_2^-$ were also determined in presence of astaxanthin (10^{-7}–10^{-5} M), SOD or quercetin (5×10^{-5} M).

Results: Incubation with pyrogallol decreased ACh-induced maximum relaxation from 78.8 × 4.7% to 19.3 × 3.1% and diminished sensitivity to SNP, as pD2 values show (from 7.5 to 7.0), without modifying maximum response. SOD completely reverted both signals of vascular damage thus indicating the involvement of $^{\cdot}O_2^-$. Astaxanthin failed to prevent neither dependent nor independent endothelium impairment.

The $^{\cdot}O_2^-$ produced by pyrogallol (1611 × 59 RLU/min) decreased in presence of SOD (598 × 19 RLU/min) or quercetin (1258 × 30 RLU/min) but not after astaxanthin incubation. However, the $^{\cdot}O_2^-$ production stimulated by β-NADH was inhibited by astaxanthin ($CI_{50} = 1.5 \times 10^{-6}$ M).

Conclusion: Our results suggest that astaxanthin lacks $^{\cdot}O_2^-$ scavenging activity but it is able to reduce the $^{\cdot}O_2^-$ generated by NADH-oxidase through mechanisms that need further investigation.

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C086
ANTITHROMBIN MODULATES AGIOGENIC GROWTH AND CONTRACTILE TONE OF RAT AORTA

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AT is a plasma protein, synthesized mainly by the liver, although AT is expressed also at minor extent in vessels and endothelial cells. Low plasma levels of Antithrombin (AT) before and during cardiac surgery with cardiopulmonary bypass are associated with a poor response to heparin, and related to a higher incidence of thromboembolic events and prolonged intensive care unit stay.

Objective: To study the role of AT on vascular tone and angiogenesis.

Aortas from Wistar rats were sectioned into 1 mm rings and seeded on 50 µl of polymerized Matrigel in a 96 well plate, with fetal calf serum supplemented endotelial basal medium- MV2, and incubated at 37°C and 5% CO_2 for 7 days. Image acquisition of tube formation was achieved daily with an inverted microscope (objective 2.5×). Contractile responses to cumulative concentrations of Phenylephrine and vasodilator responses to cumulative concentrations of A were performed in isolated rat aorta.

Addition of cumulative doses of AT (0.005–1 UI/ml) produces a concentration dependent relaxation of pre-contracted rat aorta. Maximal relaxation achieved by AT 1 UI/ml was 28.27 ± 5.57% ($n = 8$). This relaxation was not observed in presence of L-NAME 100 µM. AT 0.5 and 1 UI/ml increase the length of the new vessels formed during the angiogenic process.

AT increases angiogenic growth and modulates vascular tone through NO release. These actions could contribute to the deleterious effect of low levels of AT after cardiopulmonary bypass

C089
CARVEDILOL MODULATES ANGIOGENESIS IN HYPERTENSION AND HEART FAILURE

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Carvedilol (CV) is a third-generation β-blocker (non-cardioselective vasodilator) which maintains cardiac output, has a reduced prolonged effect on heart rate, and diminishes blood pressure by favoring the nitric oxide bioavailability and decreasing vascular resistance.

Objectives: The aim of the present study was to assess the effect of CV on hypertensive aged rats in order to: (i) assess the influence of CV on vascular contractility; (ii) determine its ability to reverse the alterations caused by hypertension (HT); (iii) analyze its effect on angiogenesis.

SHR rats (52 weeks old) were treated with CV 10 mg/kg for 5 weeks by oral gavage and compared with un-treated controls ($n = 10$ each group). Aortic rings were seeded on 50 µl of MatrigelTM in a 96 well plate and cultured in EGM-2 MV medium. During the incubation for 7 days (37°C and 5% CO_2), the length of the new vessels formed was quantified. Contractile responses to cumulative concentrations of α1-AR agonist (Phenylephrine) and vasodilator responses to cumulative concentrations of β-AR agonists (non-selective β-agonist Isoprenaline, β2-selective agonist Salbutamol and β3-selective agonist SR58611A), Acetylcholine and Sodium Nitroprusside (NO donor) were performed in isolated rat aorta.

Angiogenic growth was significantly increased after chronic treatment with CV. CV treatment also improved the vasodilatation induced by β-agonists, acetylcholine and sodium nitroprusside, as well as reduced cardiac hypertrophy secondary to hypertension in aged animals.

CV improved angiogenesis and vasodilator responses mediated by NO or β-adrenoceptors in rat aorta from aged hypertensive animals, suggesting an improvement of the endothelial function and the NO bioavailability.

C090
EFFECT OF AGE IN THE LOSS OF CONTRACTILE RESPONSE INDUCED BY PHENYLEPHRINE IN ISOLATED RAT THORACIC AORTA

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We have demonstrated a loss in the successive phenylephrine (α1-adrenoceptor agonist)-induced contractile response in rat aorta that depends on neuronal nitric oxide synthase (nNOS) (1). In this work we investigated the effect of age and hypertension on this phenomenon.

Functional studies were done in organ bath using is olated aorta from adult (16 weeks) and old (52 weeks) male Wistar and spontaneously hypertensive rats (SHR) to determine the contractile response to phenylephrine: two consecutive concentration–response curves (CRC1 and CRC2) were obtained in the absence or presence of S-methyl-L-thiocitrulline (SMTC, 1 µM, selective nNOS inhibitor). The relaxant responses to acetylcholine or sodium nitroprusside were also analysed.

The maximal contraction to phenylephrine in the CRC2 was significantly reduced respect to the CRC1 in adult Wistar rats (55.1 ± 4.0% vs. 97.9 ± 4.5% of KCl 80 mM $n = 8$, $P < 0.001$) and SHR (42.4 ± 5.5% vs. 72.0 ± 7.6% of KCl 80 mM $n = 8$, $P < 0.01$); in the presence of SMTC there was not significant differences between CRC1 and CRC2 in both groups of animals. In old Wistar rats and SHR the loss of contractile response disappears and no significant differences between CRC1 and CRC2 were found. In adult and old Wistar rats the relaxant response to acethylcholine and nitroprussiate were similar suggesting that the eNOS/NO/cGMP pathway was not altered with age and therefore confirming the involvement of nNOS but not eNOS in the loss of contractile response in adult animals.

C091
MODULATORY ROLE OF ENDOTHELIAL NEUROTROPHIN-3 ON β3-ADRENOCEPTORS IN MOUSE AORTA

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To analyze the expression of neurotrophin-3 (NT-3) and its receptor TrkC in the cardiovascular system and the role of endothelial NT-3 in vessels from genetically engineered mice.
Western Blot experiments were performed to quantify the expression levels of NT-3 and TrkC in aorta and left ventricle from Wistar rats. Functional studies were done using isolated aorta from genetically engineered mice in which the expression of endothelial NT-3 is deleted (Ntf3flox1/flox2; Tie2-cre+/0) and their controls (Ntf3flox1/flox2; Tie2-cre0/0) to determine the contractile response to phenylephrine (α1-adrenoceptor agonist). Two consecutive concentration–response curves were obtained. Endothelium was confirmed by addition of acetylcholine. Concentration–response curves of relaxation on phenylephrine-induced contractions were performed with two β-adrenoceptor agonists: isoprenaline (non-selective) and SR58611A (β3-selective).
In the left ventricle, Western blot analysis showed a stronger TrkC signal (75 kDa) than in aorta. Conversely, the NT-3 band (32 kDa) was stronger in aorta than in ventricle. Phenylephrine-induced contraction and isoprenaline-induced vasodilatation were similar in both strains. In control mice, SR58611A showed biphasic relaxation curves (pEC50high: 8.35 ± 0.30, pEC50low: 4.26 ± 0.14, β3-AR high affinity sites: 16.73 ± 3.32%, $n = 6$). In Ntf3flox1/flox2; Tie2-cre+/0 mice, SR58611A relaxed monophasically with low pEC50 (4.05 ± 0.12, $n = 2$), suggest ing a lack of β3-AR participation.
NT-3 and TrkC are both present, but differently expressed, in aorta and ventricle from Wistar rats. The lack of endothelial NT-3 expression decreases β3-adrenoceptor-mediated relaxation in mouse aorta.
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C106
ANGIOTENSIN-(1–7) MITIGATES VASCULAR INFLAMMATION AND SENESCENCE THROUGH MAS RECEPTOR

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Angiotensin (Ang)-(1–7) is a renin angiotensin system (RAS) peptide acting on the G protein-coupled receptor Mas, acting as a physiological antagonist of Ang II. We tested whether Ang-(1–7) exhibits vascular anti-inflammatory and anti-senescence actions. In cultured vascular smooth muscle cells (HASMC), the inflammatory markers used were inducible nitric oxide synthase (iNOS) levels and NO release. Pre-incubation with Ang-(1–7) (1 nM to 1 μM) reduced concentration-dependently the iNOS induction and the NO release stimulated by Ang II (100 nM; 18 h) but also by the RAS-independent pro-inflammatory cytokine interleukin (IL)-1b (2.5 and 10 ng/ml; 18 h). The anti-inflammatory effects of Ang-(1–7) were totally prevented by t he Mas receptor antagonists A779 (1 μM) and D-Pro-Ang (1–7) (1 μM). By using apocynin (30 μM) and pyrrolidine dithiocarbamate (100 μM), we established that the anti-inflammatory actions of Ang-(1–7) relied on a sequential inhibition of NADPH oxidase and nuclear factor-κB activation stimulated by Ang II or IL-1b. In human umbilical vein endothelial cells (HUVEC), the inflammatory markers used were the expression of ICAM-1 and VCAM-1 and the *in vitro* adhesion of HL60 leukocytes to a HUVEC monolayer, while senescence was determined by positive senescence-associated b-galactosidase (SA-b-gal) cell staining and by the degree of DNA damage through indirect immunofluorescence. In HUVEC, Ang-(1–7) markedly attenuated the inflammation and the senescence induced by Ang II or IL-1b, through a mechanism sensitive to the Mas receptor antagonist A779. In conclusion, the Ang-(1–7)/Mas receptor axis arises as a potential therapeutic target for modulating inflammation and senescence in the context of vascular diseases.

C108
ESTRADIOL REGULATES MIRNA PROFILE IN HUMAN ENDOTHELIAL CELLS

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Estrogens have an important role in the regulation of endothelial function. MicroRNAs (miRNAs) are a small non-coding RNAs that modulate post-transcriptional expression of numerous genes implicated in a wide range of biological processes. Our aim was to determinate the effect of estadiol on miRNA expression profile in cultured human endothelial cells.
Primary HUVEC were exposed to 1 nM estradiol for 24 h and miRNAs were isolated by miRNeasy Mini Kit (Qiagen). miRNAs expression was performed with GeneChip miRNA 4.0 Array (Affymetrix). Global differences were measured by Principal Components Analysis (PCA) and changes in the expression profile were analyzed by Hierarchical Cluster using Partek Genomic Suite v6.6 software (Partek Inc., ST Louise, MO). miRNA-gene interactions and gene ontology pathways analysis were computationally predicted using DIANA tools web service.
Global interrelationships among samples studied by PCA analysis shown differences between samples from control and estradiol-treated cells. We identified 120 miRNAs with significant differential expression, 47 up-regulated and 73 down-regulated. Putative targets of differentially expressed miRNAs revealed significant KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways relevant for endothelial function, including PI3K-AKT, MAPK and actin cytoskeleton signalling, among other pathways.
Estradiol-induced changes on miRNA expression profile adds a new level for modulation and regulation of endothelial functions. Identification of miRNA modulated by estradiol will lead to propose new pharmacological targets

C110
THE ADIPOKINE SOLUBLE DIPEPTIDYL PEPTIDASE-4 IMPAIRS MICROVASCULAR REACTIVITY THROUGH PROTEINASE-ACTIVATED RECEPTOR-2 AND THROMBOXANE A2 PRODUCTION

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Dipeptidyl peptidase-4 (DPP4) is a key protein in glucose homeostasis and a pharmacological target in type 2 diabetes mellitus. This study explored whether the novel adipokine soluble DPP4 (sDPP4) can cause defective vascular reactivity, an early marker of endothelial dysfunction and vascular complications. Reactivity was studied in isolated second-branch mesenteric arteries from 3 month-old female C56/BL6 mice, using a small vessel myograph. Neither the contractility to noradrenaline (NA; 1 nmol/l to 3 μmol/l) nor the endothelium-independent

relaxations induced by sodium nitroprusside (1 nmol/l to 100 μmol/l) were modified by sDPP4 (20–500 ng/ml). However, sDPP4 impaired in a concentration-dependent manner the endothelium-dependent relaxation elicited by acetylcholine (ACh; 1 nmol/l to 10 μmol/l). The DPP4 inhibitors K579 (100 nmol/l) and linagliptin (1–100 nmol/l) prevented the defective relaxation induced by sDPP4, as did the protease-activated receptor 2 (PAR2) inhibitor GB83 (10 μmol/l). Downstream of PAR2, similar results were obtained with the nonspecific cyclooxygenase (COX) inhibitor indomethacin (10 μmol/l), the COX2 inhibitor celecoxib (3 μmol/l), or the TP receptors blocker SQ29548 (100 nmol/l). Consistently with these results, sDPP4 triggered the release of thromboxane A2 (TXA2) by cultured endothelial cells, as determined by EIA. TXA2 release was prevented through DPP4 and PAR2 inhibition or the blockade of the COX pathway. In summary, these findings reveal sDPP4 as a direct mediator of endothelial dysfunction, acting through PAR2 activation and the release of vasoconstrictor prostanoids. By interfering with these actions, DPP4 inhibitors might help preserving endothelial function in the context of cardiometabolic diseases.

C113
ROLE OF AGEING AND ESTROGEN WITHDRAWN ON SUPEROXIDE PRODUCTION INDUCED BY THROMBOXANE A2 RECEPTOR STIMULATION

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Aging is a main cardiovascular risk factor related in part with an increased oxidative stress. Thromboxane A2 (TXA2), a pro-oxidant and pro-inflammatory endogenous vasoconstrictor, is also associated with endothelial dysfunction. We studied the role of superoxide in the vascular response mediated by TXA2 and the contribution of ageing and estrogen withdrawn, both conditions present in menopause.

Five-months-old female Senescence-Accelerated Mice (SAM)-Resistant (SAMR1; $n = 15$) and SAM-Prone (SAMP8; $n = 15$) were sham-operated, ovariectomized or ovariectomized treated with estradiol. 28 days after surgery, aorta was excised and incubated with U46619, a stable analog of TXA2 (10^{-8} M) in the presence and in the absence of 4-hydroxy-TEMPO (10^{-3} M), TXA2 receptor (TP) antagonist GR-32191B (10^{-8} M) and indomethacin (10^{-5} M). Vascular superoxide levels were detected by dihydroethidium staining on sections of thoracic aorta through a fluorescent microscope. Analysis was performed using Image J software and data are expressed as average of fluorescence.

U46619 increased the superoxide production in aorta from SAMR1 and SAMP8 mice. Ovariectomy boosted the superoxide production in SAMR1, increase that was higher in SAMP8 ($78 \pm 5\%$ vs. $157 \pm 7\%$; $P < 0.05$). These rises in superoxide levels were reversed by estrogen treatment (10 μg/kg/day 17b-estradiol). Treatment with 4-hydroxy-TEMPO, GR-32191B and indomethacin prevented the increase in superoxide formation induced by U46619.

The superoxide generation in response to TP-receptor stimulation is enhanced in ovariectomized mice and further increased in senescent mice. This effect is mediated by cyclooxigenases activation.

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C119
FRACTALKINE (CX3CL1) IS INVOLVED IN TNFALPHA-INDUCED PLATELET-LEUKOCYTE ADHESION TO THE ARTERIAL ENDOTHELIUM IN YOUNG PATIENTS WITH IDIOPATIC VENOUS THROMBOSIS

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The inflammatory status in idiopatic venous thrombosis (VTE) in young patients and its association to the development of further cardiovascular disorders has been barely investigated. Therefore, the potential link between CX3CL1/CX3CR1 axis in VTE and endothelial dysfunction was investigated.

Whole blood from 22 VTE individuals and 23 age-matched controls was analysed by flow cytometry. CX3CR1 expression on different leukocyte subsets was evaluated. Parallel-plate flow chamber assay was employed to evaluate platelet-leukocyte and leukocyte adhesion to TNFα (20 ng/ml)-stimulated human umbilical arterial and venous endothelial cells (HUAEC and HUVEC, respectively).

VTE patients presented greater expression of CX3CR1 on circulating platelet-monocyte aggregates compared with age-matched controls. Increased adhesion of platelet-leukocyte aggregates to TNFα-stimulated HUAEC was found in VTE patients vs. age-matched controls. Neutralization of CX3CL1 activity significantly inhibited TNF-induced platelet-leukocyte-HUAEC interactions (35% inhibition) in VTE patients but not in aged-matched controls. Platelet-leukocyte or leukocyte adhesion to TNFα-stimulated HUVEC was found to be CX3CL1 independent in HUVEC. Plasma levels of CX3CL 1 were significantly increased in VTE patients compared to aged-matched control.

We provide the first evidence that increased CX3CR1 expression on circulating platelet-leukocyte aggregates from VTE patients may constitute a prognostic marker of adverse cardiovascular events in this pathology. Consequently, CX3CL1/CX3CR1 axis blockade might be a new therapeutic target in the prevention and treatment of VTE-associated cardiovascular disorders. This study was supported by grants SAF2011-23777, CPII13/00025, and PI012/01271 from the Carlos III Health Institute, the Spanish Ministry of Economy and Competiveness, and FEDER.

C127
EFFECT OF SITAGLIPTIN ON ENDOTHELIAL PROGENITOR CELLS IN CULTURE

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In patients with cardiovascular disease (CVD) the functionality of EPCs is poor, although the exact mechanism of dysfunction is still uncertain. The SDF1/CXCR4 axis have become a key element in the study of CVD. Recent studies have postulated a beneficial effect of DPP4 inhibitors in the circulating EPCs. Our main objective was to determine the effect of Sitagliptin on the expression of SDF1/CXCR4 in EPCs, since it is one of the natural substrates of DPP4.

The effect of Sitagliptin (1, 2 and 5 μM at 6, 12 and 24 h) on the expression of SDF1/CXCR4 on EPCs in culture were measured by WB and ELISA.

It was observed that Sitagliptin 1 μM in EPC improves cell morphology an d increases the formation of colonies at 6, 12 h ($P < 0.001$). Similar effect was observed with Sitagliptin 2 μM at 12 h. Moreover, was observed an increased levels of SDF-1α with Sitagliptin 1 and 2 μM with significant trend at 24 h. The effect in the expression of CXCR4 was higher ($P = 0.01$) at 24 h with Sitagliptin 1 μM, and the similar effect was observed with Sitagliptin 2 μM at 12 h (NS).

Sitagliptin would help reverse the poor functionality of the EPCs, by means of modulation of SDF1/CXCR4 axis.
Acknowledgements: FIS PI12/00590 (planestatal I + D + I 2013–2016).

C128
EFFECT OF SITAGLIPTIN (INHIBITOR OF DIPEPTIDYL PEPTIDASE 4) ON THE PROLIFERATION AND APOPTOSIS OF VASCULAR SMOOTH MUSCLE CELLS OF DIABETIC AND NON-DIABETIC PATIENTS

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Sitagliptin is a drug used for the treatment of the diabetes. It is a selective inhibitor of dipeptidil peptidasa 4 (DPP-IV). It has been reported that DDP-IV would lead to NFκβ activation, which is a regulator of the vascular smooth muscle cells (CMLV) proliferation. We investigated the effect of sitagliptin on the proliferation and apoptosis of CMLV of diabetic patients (D) and non-diabetics patients (ND).
The CMLV were obtained of mesenteric arteries from abdominal surgery of diabetic patients and non-diabetics patients, and cultivated with sitagliptin in increasing concentrations (1, 2, 5, 25, 50 and 100 μM). For the study of cell viability the cell count was performed to the 24 h, 72 h, 7–14 days. Cellular proliferation was assessed by a BrdU incorporation kit. Apoptosis was measured by DNA fragmentation ELISA ($n = 3$).
In non-diabetic patients there were a decrease in the number of cells (cells counter) at 72 h in the concentrations of sitagilptin of 25, 50 and 100 μM ($P < 0.0001$). Morover the apoptosis increased significantly to the concentrations 5–100 μM ($P < 0.0001$). Cellular proliferation (BrdU incorporation) decreased gradually at all concentrations, being more pronounced at higher concentrations (50 and 100 μM, $P < 0.001$). In diabetic patients sitagliptin causes a significant decrease in the number of cells in all concentrations at 24 h. The apoptosis significantly increases and the proliferation diminishes significantly in all drug concentrations ($P < 0.0001$).
Sitagliptin diminishes the cellular proliferation and increases apoptosis both in cells of diabetic and non-diabetic patients, being these effects more pronounced in patient diabetics.

C130
ETS-2 A POSSIBLE MARKER OF EARLY INSTABILITY IN CABG PATIENTS

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In patients with cardiovascular disease (CVD), the endothelial progenitor cells (EPCs) play a key role in endothelial repair processes. It has hypothesized that Ets2 transcription factor could be involved so active in the instability of CVD/hyperlipidemia. Our main objective was to determine the degree of expression of Ets-2-endoglin in peripheral blood mononuclear cells and relate the data found with clinical parameters from patients undergoing coronary artery-bypass grafting (CABG).
Seventy patients undergoing CABG from the cardiac surgery service were selected for the study and categorized into five CVD stages. The Expression of Ets-2, CXCR4, and endoglin were measured by Western blot.
It was observed an increase in the expression of the transcription factor Ets-2, in patients without CV predictor risk factor associating with early stages of CV instability (GI: 2.16 ± 1.4), and its expression was decreased in patients with longer evolution of CVD (GII: 1.16 ± 0.8; GIII: 1.06 ± 0.5; GIV: 0.91 ± 0.3; GV: 0.56 ± 0.3). It was found a direct association of expression of Ets-2 with age ($P = 0.04$) and endoglin expression ($P = 0.008$), and indirectly with the evolution of CVD ($P = 0.008$) and hyperlipidemia ($P = 0.03$). The silent of expression of Ets-2 in EPCs in culture by SiRNA, decreased the expression of endoglin ($P = 0.001$).
The transcription factor Ets-2 could be an early marker of cardiovascular instability associated with states of hyperlipidemia. Our data suggesting that a poor functionality of circulating EPCs could be associated with decreased expression of the transcription factor Ets-2 in advanced stages of cardiovascular disease.
Acknowledgements: FIS P I12/00590 (planestatal I + D + I 2013–2016).

C132
PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR β/δ ACTIVATION RESTORES THE PALMITATE -INDUCED IMPAIRMENT OF INSULIN SIGNALING IN ENDOTHELIAL CELLS

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Obesity, type 2 diabetes, and metabolic syndrome are associated with elevated circulating concentrations of free fatty acids (FFAs). Elevated FFAs concentration in circulation is widely accepted as an independent risk factor and has been proven to impair nitric oxide (NO) production of endothelial cells by inducing chronic inflammation and oxidative stress.
The current study aimed to determine whether PPARβ/˙ may independently improve the endothelial dysfunction caused by high FFAs and the underlying mechanism.
Human umbilical vein endothelial cells (HUVECs) were incubated with the GW0742 or plus GSK0660, during 12 h, and in the last 3 h in medium 199 containing BSA (control cells) or palmitate (100 μM)-conjugated BSA. Cells were then used to measure NO or reactive oxygen species (ROS) production, NADPH oxidase activity, gene and protein expression. Palmitate reduced insulin-stimulated NO, and increased ROS production in HUVECs. Palmitate also reduced insulin-induced Akt-Ser473 and eNOS-Ser1177 phosphorylation. GW0742 increased NO production through the up-regulation of AKT/eNOS signaling and suppressed ROS generation. PPARβ/˙ activation also prevented NADPH oxidase activity induced by palmitate. Gene expression profiling revealed a marked increase of the carnitine palmitoyl transferase-1 (CPT-1) and uncoupling protein-2 (UCP2) in GW0742 treated HUVECs. GW0742-induced effects were abolished by GSK0660 or the CPT-1 inhibitor etomoxir and UCP-2 inhibitor genipin.
PPAR-β activation *in vitro* restores the endothelial function, preserving the insulin-Akt-eNOS pathway impaired by palmitate, at least in part, through CPT-1 and UCP-2 activation.
Supported by SAF2014-55523-R, P12-CTS-2722 and RD12/0042/0011.

C133
RICE BRAN ENZYMATIC EXTRACT DIET SUPPLEMENTATION REDUCES OXIDATIVE STRESS AND INFLAMMATION ASSOCIATED TO ATHEROSCLEROSIS DISEASE IN APOE$^{-/-}$ MICE

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Atherosclerosis oxidative stress and inflammation are key factor in the onset and progression of atherosclerosis. High content of γ-oryzanol, phytosterols and tocols in rice bran provide interesting lipid-lowering, antioxidant and anti-inflammatory effects. Herein, we aimed to study the effects of rice bran enzymatic extract (RBEE) on the inflammatory and prooxidant state associated to atherosclerosis disease.

7 week-old ApoE$^{-/-}$ mice were fed high fat diet (HFD) supplemented or not with 1 or 5% RBEE (w/w) for 23 weeks. 14 μm slices from the aortic arch were used to measure stenosis. NF-κβ location was assessed by ELISA. Inflammatory markers TNF-α, iNOS and COX-2 and NADPHox subunits Nox-1, p22phox and p47phox protein expressions were measured by Western Blot from whole aorta homogenates. Dihydroethidium fluorescence and iNOS immuno-staining were evaluated by confocal laser scanning from aortic arch slices.

RBEE diet supplementation reduced plaque burden ($P < 0.001$) induced by HFD. HFD increased nuclear p65 NF-κβ subunit ($P < 0.05$) and induced expression of proinflammatory cytokines and enzymes TNF-α, iNOS and COX-2 ($P < 0.001$). RBEE diet reduced p65 nuclear traslocation ($P < 0.05$) and downregulated iNOS expression in the aortic arch ($P < 0.05$). Increased superoxide production was observed in animals fed HFD ($P < 0.001$). 1% and 5% RBEE diet supplementation reduced oxidative stress by reducing Nox-1 and p47phox expression ($P < 0.05$). Additionally, 1% RBEE supplements reduced p22phox expression ($P < 0.01$), resulting in superoxide anion release blockage ($P < 0.5$).

Regular RBEE consumption reduced inflammation and oxidative stress associated to atherosclerosis disease, showing its potential as functional food in dyslipidemia related diseases.

C137
BENEFICIAL EFFECT OF LYCOPENE IN OXIDATIVE STRESS INDUCED BY ANGIOTENSIN II *IN VIVO*

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The aim of this study was to investigate whether treatment with lycopene influence the oxidative stress caused by angiotensin II (Ang II) in rats and thus justify the improvement in hypertension that we previously reported (1).

Wistar rats were infused for 14 days with Ang II (288 mg/kg/day) using osmotic minipumps implanted subcutaneously (ANG). Half were treated simultaneously with lycopene (10 mg/kg/day, p.o.; LYC). Sham rats were used as control (Control). Systolic blood pressure was monitored by the tail cuff-method. MDA as assessment of lipid peroxidation in plasma, $O_2^{\cdot -}$ production by lucigenin-enhanced chemiluminescence in aorta, superoxide dismutase (SOD) and catalase (CAT) activity in liver are determined as oxidative stress markers.

The endothelial function was tested by performing concentration-response curves to acetylcholine (ACh) in aortic rings and perfused kidney.

Lycopene treatment attenuated blood pressure increased by Ang II and improved the response to ACh in perfused kidney but not in aorta.

MDA and $O_2^{\cdot -}$ levels increased when animals received Ang II (ANG, 3.7 ± 0.2 vs. Control, 2.9 ± 0.1 nmol/ml and ANG, 49578 ± 4104 vs. Control, 33915 ± 3980 RLU/min, respectively) and treatment significantly reduced these levels (LYC, 3.1 ± 0.1 nmol/ml and 33481 ± 6635 RLU/min). Lycopene increased significantly the SOD, but did not modify CAT activities.

Our results show that lycopene have antioxidant activity '*in vivo*' related to their ability to improve lipid peroxidation damage and antioxidant enzymes activity. This along with its effect in $O_2^{\cdot -}$ production could explain the beneficial effect on endothelial dysfunction and hypertension.

Lycopene was kindly supply by DSM NUTRICIONAL PRODUCTS (México).

(1) Santos, P.; Sevilla, M.A.; Carrón, R.; Monroy-Ruiz, J.; Montero, M.J.: Licopeno previene la hipertensión y la hipertrofia cardiovascular producida porangiotensina II. Actualidad en Farmacología y Terapéutica, 2013; 4:179–181

PHARMACOGENETICS AND PHARMACOGENOMICS

C006
POWERFUL PHARMACOGENETIC PREDICTOR OF EXTRAPYRAMIDAL SYMPTOMS INDUCED BY ANTIPSYCHOTICS: MULTILOCUS INTERACTION IN THE MTOR PATHWAY

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Antipsychotic (AP) treatment-emergent extrapyramidal symptoms (EPS) are acute adverse reactions of APs. Recent studies showed that dyskinesia induced by L-Dopa in the treatment of Parkinson's disease may be related to sensitized DRD1 signaling in the direct pathway which involves the activation of the mammalian target of rapamycin kinase (mTOR) pathway. The aim of the present study is to analyze gene–gene interactions in nine genes related to the mTOR pathway, in order to develop genetic predictors of the appearance of EPS.

A total of 243 subjects (78 presenting EPS: 165 not) from three cohorts participated in the present study: Cohort 1, patients treated with risperidone, (n = 114); Cohort 2, patients treated with APs other than risperidone (n = 102); Cohort 3, AP-naïve patients with first-episode psychosis treated with risperidone, paliperidone or amisulpride, n = 27. Selected Polymorphisms (rs7874234 (TSC1), rs13335638 (TSC2), rs2024627 (mTOR), rs1130214 (AKT1), rs456998 (FCHSD1), rs1801582 (PARK2), rs3737597 (DISC1), rs7211818 (Raptor) and rs1053639 (DDIT4)) were detected by real-time PCR.

In Cohort 1, we identified a four-way interaction, including the rs1130214 (AKT1), rs456998 (FCHSD1), rs7211818 (Raptor) and rs1053639 (DDIT4), that correctly predicted 97 of the 114 patients (85% accuracy). We validated the predictive power of the four-way interaction in Cohort 2 and in Cohort 3 with 86% and 88% accuracy respectively.

The inclusion of this predictor in routine clinical practice could have important benefits for clinicians, patients and the healthcare system as a whole.

Patent has been applied for (EP13382027) and commercialized by AB-Biotics 'Neuropharmagen' Published in Eur Neuropsychopharmacol, 2015; 25: 51–59.

C121
EFFECTS OF GENE POLYMORPHISM OF CYP3A5 6895A>G GENOTYPES ON DONORS AND RECIPIENTS OVER CHRONIC NEPHROPATHY IN ADULTS LIVER TRANSPLANT RECIPIENTS TREATED WITH TACROLIMUS. COHORT STUDY

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Calcineurin inhibitors (CI) metabolites can greatly exceed CI levels in patients blood and tissue and can be toxical potencial. The expressers polymorphisms of its metabolize enzime gene (CYP3A5 6986A>G) have been associated with higher metabolites concentration and risk of chronic CI nephrotoxicity (CN) compared with non-expressors (CYP3A5^3/^3). But there are studies with opposite results.

Evaluate the impact of polymorphism of CYP3A5 6895A>G genotypes of donors and recipients over CN in adults liver transplant recipients treated with tacrolimus. Methods: We included patients who were showed follow-up time at least 3 years post-transplantation. CN were diagnosed based on clinical criteria. For contrast the genotypes with the outcomes, we built two groups: Expresser: CYP3A5*1. Non-expresser: CYP3A5*3/*3. We performed multivariable regression to assess the contribution of clinical variables.

77 patientes were included. Allele frequencies were as follows: CYP3A5 genotypes, the *1/*1, *1/*3, and *3/*3 genotypes were detected in 4, 12, and 61 of donors, and 3, 10 and 64 of recipients, respectively. The incidence of CN was affected by the CYP3A5*1 allele in donors (expresser vs. no-expressers: 37.5% vs. 11%, P = 0.01.; OR 4.2, 95% CI 1.02 to 19.8). This effect is higher in recipients with CYP3A5 genotypes expresser.

CYP3A5*3 /*3 genotype of donors provide more susceptible for developing CN associated with tacrolimus. This effects is more pronounced in recipients expressers. It could be the result of toxic action of tacrolimus metabolites in renal tissue secondary a higher concentrations and metabolism of this drug.

NEUROPSYCHOPHARMACOLOGY

C009
EVALUATION OF A NEURODEGENERATION ANIMAL MODEL BY THE MORPHOMETRIC APPROACH WITH MAGNETIC RESONANCE IMAGING

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Insulin and IGF-I exert various physiological effects by activating insulin receptor substrate (IRS) proteins. The deletion of IRS-2 in mice reduces the brain size due to impaired neuronal proliferation during development, with hyperphosphorylation and accumulation of the microtubule-associated protein tau in the adult. Indeed, IRS-2 deficiency might represent a reasonable model of neurodegeneration. The formation of plaques and tangles, and inflammation in neurodegenerative diseases include loss of neurons; however, diagnosing these pathological changes by histological methods is only feasible post-mortem. Therefore, the *in vivo* visualization of cerebral pathophysiology would facilitate the disease staging and pharmacotherapy in humans, and would enable animal models of neurodegenerative disorders.
Magnetic resonance imaging (MRI) was used as an analytical tool for characterizing *in vivo* neurodegeneration in 18-months-old mice, WT and IRS-2 knocked-out (KO). Afterwards, the animals underwent a post-mortem immunohistochemical study.
The MRI analysis revealed a very striking pathological difference in cerebral ventricles of aged KO mice contrasting with WT controls. By measuring the area, diameters and perimeter of lateral, medial and fourth ventricles, significant increases of these structures in the brains of KO was observed. Interestingly, enlarged ventricles have been reported for several neuronal disorders including schizophrenia and Alzheimer disease. Subsequent histological analysis of those brains revealed neuronal loss and amyloid deposit s, together with reduction in the thickness of cortical areas and bas al ganglions.
These observations suggest that MRI provides a valuable tool for *in vivo* detection of pathological changes in brains of mouse models suffering neurodegeneration processes.

C018
CXCR6/CXCL16 AXIS MEDIATES PLATELET-LEUKOCYTE ADHESION TO THE DYSFUNCTIONAL ARTERIAL ENDOTHELIUM IN COPD

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Cardiovascular disease (CVD) is a major co-morbidity in chronic obstructive pulmonary disease (COPD), yet the pathways involved in its development remain unknown. Therefore, the potential link between CXCR6/CXCL16 axis and COPD-induced endothelial dysfunction were investigated.
Whole blood from 21 COPD patients and 15 age-matched controls was analysed by flow cytometry. Platelet activation (P-selectin expression and circulating PAC-1$^+$ platelets) and CXCR6 and CXCL16 expression was determined. CXCR6 expression on different leukocyte subsets was also evaluated. Parallel-plate flow chamber assay was employed t o evaluate platelet-leukocyte and leukocyte adhesion to cigarette smoke extract (CSE)-stimulated arterial endothelium.
Flow cytometry analysis revealed that COPD patients presented greater numbers of activated circulating platelets (PAC-1$^+$) with increased expression of P-selectin, CXCL16 and CXCR6 compared with age-matched controls. Additionally, COPD patients presented augmented numbers of platelet-neutrophil, platelet-monocyte and platelet-lymphocyte aggregates than age-matched control subjects. This correlated with enhanced platelet-leukocyte and leukocyte adhesiveness to CS extract (CSE)-stimulated arterial endothelial cells and was partly dependent on endothelial CXCL16 up-regulation and increased CXCR6 expression on platelets and leukocytes.
We provide the first evidence that increased CXCR6 expression on circulating platelets and leukocytes from COPD patients may constitute a prognostic marker for adverse cardiovascular events.

C023
REPEATED SOCIAL DEFEAT STRESS INCREASES THE REWARDING EFFECTS OF COCAINE AND THE LEVELS OF THE BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) IN DENTATE GYRUS IN ADOLESCENT AND ADULT MICE

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Drugs of abuse act as reinforces influencing learning and memory processes which results in long-term memory of drug reward. Therefore, social defeat stress increases the rewarding effects of psychostimulant drugs such as cocaine. Based on this, we have evaluated the effects of repeated social defeat (RSD) on the conditioned rewarding effects of cocaine using conditioned place preference (CPP) paradigm. Adolescent (PND 27–36) and adult (PND 47–56) mice were exposed to four episodes of social defeat and were conditioned 3 weeks later with cocaine (1 mg/kg, i.p.). The effects of social defeat on the brain-derived neurotrophic factor (BDNF), molecular mediator of memory consolidation processes, Met-enkephalin and pCREB in the dentate gyrus were also evaluated. Adolescent and adult mice exposed to RSD showed an increase in the conditioned reinforcing effects of cocaine and displayed higher levels of BDNF and pCREB in the dentate gyrus when compared to controls. However, Met-enkephalin levels only were increased in adolescent defeated mice. These results support the idea that RSD stress increases the rewarding effects of cocaine in parallel with increased BDNF and pCREB levels in the dentate gyrus, hippocampal region known to contribute to the formation of memories. Thus BDNF could be implicated in the increase of the reinforcing effects observed in mice treated with cocaine and exposed to RSD.

C030
ROLE OF THE INSULIN RECEPTOR SUBSTRATE TYPE 2 (IRS2) FOR MAINTAINING THE HIPPOCAMPAL INTEGRITY AND TO AVOID NEURODEGENERATION

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The insulin receptor substrate (IRS) integrates signals from the receptors of insulin and IGF-1, by linking physiological intracellular path-

ways that control growth and cell survival. The maturation and survival of the retinal photoreceptors immediately after birth is promoted by the action of IRS2 protein within the nervous tissue. Its deletion produces additional pathology in the brain: hyperphosphorylation and accumulation of Tau microtubule-associated protein. Since the alterations in cerebral development appear prior to the onset of hyperglycemia, the dysregulation of the signaling cascade mediated by the IRS2 type could be a molecular bridge between diabetes and neurodegenerative diseases.
Brains obtained from WT and IRS2 knocked-out (KO) mice, of both gender, underwent immunohistochemical staining.
In the IRS2-KO animals, there were cerebral alterations of hippocampus at dentate gyrus and Ammon's horn (CA1, CA2 and CA3), which suggest that the IRS2 protein is essential for maintaining the structural integrity of those zones. The neural sinaptology decayed considerably as there was a marked and significant decrease in one of its indicators, the SV2 synaptic vesicle protein. Furthermore, in these animals a significant reduction in hippocampal GABAergic neurons was observed.
In the IRS2-KO animals, there were cerebral alterations of hippocampus at dentate gyrus and Ammon's horn (CA1, CA2 and CA3), which suggest that the IRS2 protein is essential for maintaining the structural integrity of those zones. The neural sinaptology decayed considerably as there was a marked and significant decrease in one of its indicators, the SV2 synaptic vesicle protein. Furthermore, in these animals a significant reduction in hippocampal GABAergic neurons was observed.

C042
IN VIVO EVALUATION OF PHTHALAZINONES AS ANTIALZHEIMER DRUGS

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The aim of this study is to evaluate the effects *in vivo* of two new phthalazinones, the NV24 and NV29 (1.5 mg/kg, i.p.), as potential drugs to improve learning/memory in mice pre-treated with scopolamine (1 mg/kg, i.p.). Previous *in vitro* studies have shown to be effective inhibitors of acetylcholinesterase (AChE) with IC_{50} values of 6.83 ± 0.46 μM and 0.90 ± 0.14 μM, for NV24 and NV29 respectively. Inhibition of AChE potentiates cholinergic neurotransmission in CNS thus improving cognitive functions and memory in patients with AD.
The object recognition test (ORT) was performed in independent square arenas, with Zones A and B, in two periods of observation of 5 min with interval repose between them, of another 5 min. In the first period the animals were exposed to same objects in zones A and B (rectangular prism) and in the second period the prism of zone B was replaced with a cylinder. Animals were treated at −30 and −15 min prior the first period of observation. Automatic evaluation of frequency in zone and other parameters was performed with the Ethovision (Noldus, The Netherlands).
In these preliminary work the new molecules, especially NV24 increased preference for new object in mice pre-treated with scopolamine. New experiments are necessary to reduce results variability with NV29.

C043
DIMERIZED PERIMIDINONES AS POTENTIAL ANTIDEPRESSANT DRUGS

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In previous investigations, simple perimidinones were assessed as potential antidepressant drugs. The results were meaningful and the experimental data and unusual outcomes were protected by Spanish and international patents.
In the current work, we have progressed in the research by the chemical dimerization of these molecules and subsequent pharmacological evaluation as antidepressants with the Porsolt test. We also determined their theoretical molecular properties with the software Molinspiration.
In the Porsolt test we measured the time of immobility, mobility and strong mobility of three dimers (DIME) by using the software Ethovision (V 3.1.16, Noldus, Wageningen, The Netherlands).
The obtained results were not significant, showing immobility values of 199.00 ± 12.93 s (DIME-1), 205.00 ± 6.46 s (DIME-2) and 212.25 ± 11.92 s (DIME-3) compared to the low value observed in the reference drug (clorgyline) 121.30 ± 5.82 s ($P < 0.05$). The study of molecular properties exhibited that the characteristics of these new molecules are very different from the clorgyline.
In conclusion, dimerization of perimidinones suppresses their pharmacological activity as antidepressants.

C049
NORADRENALINE AND SEROTONIN REGULATION OF SIRT2 AND HDAC5: FUNCTIONAL IMPLICATIONS

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Major depression disorder (MDD) is a chronic mental illness known to be triggered by genes and environment interactions. Certain environmental stimuli responsible for this illness cause functional changes in synaptic plasticity that may be mediated by epigenetic mechanisms. Scientific evidences show that antidepressants produce changes in expression levels of histone deacetylases (HDACs) superfamily that modulate histone and non-histone proteins acetylation without changing the DNA structure itself. We aimed to study the regulation of different HDAC′s by antidepressants and the functional implication using identified HDAC′s inhibitors.
Cell line SH-SY5Y cultures were treated with the antidepressants imipramine, fluoxetine and reboxetine (10 μM) as well as with the specific Sirtuin 2 (SIRT2) inhibitor (33i) and HDAC4/5 inhibitor (MC3822) at 10 μM. Mice (C57BL6, 8 weeks old) were chronically treated with imipramine (10 mg/kg/day), fluoxetine (15 mg/kg/day) and reboxetine (25 mg/kg/day) and their respective saline controls. Protein expression of selected HDACs, histone acetylation and other synaptic plasticity markers was explored by Western Blot analysis in the prefrontal cortex. Imipramine and reboxetine treatment increase HDAC5 phosphorylated form, mainly located in the cytoplasm. SIRT2 was downregulated by all monoaminergic antidepressants used both *in vivo* and *in vitro*. Furthermore, antiderpessants and MC3822 and 33i increased synaptic plasticity *in vitro*.
Our results suggest that cytoplasmatic HDAC5 export and SIRT2 downregulation induced by monoaminergic antidepressants could contribute to the well-known beneficial effects of antidepressants on brain plasticity. An important role for synaptic levels of noradrenaline and serotonin in regulating these HDACs is suggested.

C081
MALEIMIDES DERIVATIVES AS NEW PDE10A INHIBITORS OR PUTATIVE DRUGS FOR CNS DISEASES

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As long as the location of PDE10A has been described, it's potential as a therapeutic target has been gaining importance in various CNS diseases (Seegre TF. 2003). Diseases like Schizophrenia (Schmidt CJ.

2008), Parkinson's Disease or Huntington's Disease (Carmela G. 2010). For this reason and based on previous studies by the Group of Medical Chemistry and Translational Biology of CIB-CSIC and Bio-Farma Group (USC) with inhibitors compounds of PDEs, by virtual screening, three compounds derived from maleimides were selected as specific inhibitors of PDE10A as possible seeds in a program of early drug discovery. These compounds were tested as inhibitors in a battery of PDEs to define their potency and selectivity using a fluorimetric met hod (based on the polarization of fluorescent molecules) or a radiometric method (based in a proximity scintillation assay), also characterize their competition against substrate. Of these initial three compounds it was describe the Compound 1 as PDE10A selective compound (IC_{50} = 4.6 μM) and some PDE7A inhibition activity (IC_{50} = 11.9 μM), being competitive against cAMP and cGMP as PDE10A ligand. Based on these results we propose that maleimides derivatives compounds could form a family of possible PDE10A2 inhibitor drugs against diseases of the CNS.

C082
EARLY ALTERATIONS CAUSED BY THE INTAKE OF HIGH FAT DIET IN APP/PS1 MICE AS A MODEL OF FAMILIAL ALZHEIMER DISEASE

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Alzheimer disease (AD) is the most common form of progressive dementia, which affects 5–10% of the world′s population. Previous studies have demonstrated that APP/PS1 mice fed with high fat diet (HFD) present alterations in molecular mechanisms involved in AD. Thus, the main objective of this study was to evaluate the metabolic syndrome caused by the intake of HFD in 3-month-old APP/PS1 mice as a model of familial AD.

Protein levels and mRNA expression were analyzed in hippocampus by western blotting and RT-PCR, respectively. In addition, β-amyloid plaques were detected in cortex by S-Thioflavine staining.

In our work, the protein levels of α-secretase (ADAM10), enzyme involved in the non-amyloidogenic pathway of APP processing, decreased significantly in APP/PS1 HFD. In contrast, β-secretase (BACE), involved in the amyloidogenic pathway, showed a slight increase in APP/PS1 mice. Moreover, we observed the formation of β-amyloid plaques in the cortex of APP/PS1, being significantly higher in APP/PS1 HFD. Genes involved in memory processes did not show changes among different groups. Furthermore, protein levels of insulin degrading enzyme (IDE) and PGC-1α, involved in metabolic processes, were significantly reduced in APP/PS1 HFD mice, showing a similar profile to protein kinase A (PKA), phospho cAMP response element binding protein (p-CREB). However, the phospho cyclin-dependent kinase 5 (p-CDK5) was up-regulated in APP/PS1 HFD.

In conclusion, our findings showed early alterations of molecular pathways, which were enhanced in transgenic mice fed with HFD. These molecules might be considered therapeutic targets in AD.

C083
PSYCHOSTIMULANT PROPERTIES AND BEHAVIORAL SENSITIZATION FOLLOWING MDPV EXPOSURE TO RODENTS

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3,4-methylenedioxypyrovalerone (MDPV), is a synthetic cathinone which acts as a dopamine uptake blocker, being even more potent than cocaine and probably with similar abuse liability. Thus, the aim of this study was to characterize the psychostimulant and sensitizing properties of MDPV and its relation with some neurochemical markers.

Locomotor activity was recorded in rats after MDPV (0.3, 1 or 3 mg/kg) administration. After MDPV injection (0.3 mg/kg), extracellular dopamine levels in nucleus accumbens (Nacc) were analyzed by microdialysis. In sensitization experiments, MDPV (0.3 mg/kg) was administered to mice in a 5-day paradigm and its hyperlocomotion was registered. Following 10 days of withdrawal, mice were challenged with saline, MDPV (0.3 mg/kg) or cocaine (10 mg/kg). We also investigated changes in protein expression associated with sensitization such as CREB, phospho-CREB, ΔFosB, and FosB in striatum by immunoblotting.

In rats, MDPV produced a hyperlocomotion which was prevented by haloperidol (0.1 mg/kg) pretreatment. After MDPV administration, extracellular dopamine increased around 300% over basal levels in Nacc. In mice, MDPV administration induced behavioral sensitization. Interestingly, MDPV-pretreated animals also exhibited a cross-sensitized locomotor response to cocaine. While MDPV challenge produced an increase in CREB levels in all groups, phospho-CREB was only enhanced in MDPV-pretreated animals. During withdrawal and after MDPV challenge a significant increase in ΔFosB was also observed, but not in FosB levels.

To sum up, MDPV has powerful psychostimulant effects due to an increase of synaptic dopamine availability. Repeated MDPV exposure produces sensitization and cross-sensitization to cocaine, accompanied by changes in CREB, phospho-CREB and ΔFosB levels.

C096
SELECTIVE D2-LIKE DOPAMINERGIC HEXAHYDROCYCLOPENTA[IJ]ISOQUINOLINES

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Modulation of dopaminergic system via the dopamine receptors (DR), and especially D2-like DR, has been the target for drug designing applied in the treatment of psychiatric and neurological disorders. In this context, tetrahydroisoquinoline (THIQ) alkaloids are biosynthetically dopamine-derived metabolites with affinity for DR. The aim of the present work is the synthesis of new dopaminergic compounds with THIQ structure (series 1 and 2, 2, 2a–e and 5, 5a–e) and tricyclic hexahydrocyclopenta[ij]isoquinolines (HCPIQs; series 3, 3, 3a–e).

THIQs were synthesized via Bischler–Napieralski cyclization, and HCPIQs via (E)-1-styryl-THIQ intermediate by Friedel–Crafts cyclization. Then, all the synthesized compounds were tested for their ability to displace selective radioligands of D1 and D2-like DR from their specific binding sites in striatal membranes. In addition, cytotoxicity studies in human cells were carried out by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay on HUVEC.

Seven compounds displayed high affinity towards D2-DR. Among the 1-styryl-THIQs (series 1) and 1-propenyl-THIQs (series 2) the most active compounds (Ki = 72–18 nM) possessed both catechol function and N-substitution (tertiary amine: Me or allyl). However, HCPIQ showed the highest affinities (Ki = 29–13 nM) and selectivity for D2-DR, with selectivity ratios (Ki D1/D2) between ~2500 and 400. None of the most active compounds on D2-DR showed any relevant cytotoxicity.
Catechol group and N-substitution significantly modulate D2-DR affinity on THIQ structure. The presence of cyclopentane ring seems to improve both D2-DR affinity and selectivity.

C101
ROLE OF SOCIAL DEFEAT IN MORPHINE ASSOCIATED RELAPSE

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It has been shown previously that stress has a prominent role in drug addiction existing a loop between noradrenergic system and brain stress system for NA and CRF release. Also, it has been suggested that stress and different factors in daily life could influence an increase in drug-taking and the resulting addiction. This study was based on observing how different social factors could act as stress factors influencing a future relapse into drug use after a period of abstinence, including withdrawal. This research involved 20 mice divided into two groups: one corresponding to social stress stimuli (Social Defeat), and the other one was the control group. All the animals were administered an increasing dose of morphine (10, 30, 50 and 60 mg/kg) and the last day they were firstly injected with naloxone (1 mg/kg), and secondly introduced in a place-preference box in order to develop CPA. After 5–9 sessions, when the CPA was extinguished in all mice, we proceeded to perform stress tests. Our results, statistically significant, showed that physical and social stimulus were able to create the state of aversion which mice had previously faced so it could be said that a social stimuli like Social Defeat has an effect on substance abuse relapse in opioid-dependent animals.

C134
NEUROGENESIS MANIPULATION AFTER STROKE CAN AFFECT A PREVIOUS ACQUIRED MEMORY

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The contribution of hippocampal neurogenesis to the recovery of cognitive function after stroke has been previously evaluated 1–4 by manipulating neurogenesis before stroke and then, the ability to form new hippocampus-dependent memories were evaluated. The conclusion is that ablating neurogenesis after ischemia impedes cognitive recovery. Recent studies suggest that increasing neurogenesis after the formation of a memory induces forgetting 5. With this background, we asked whether manipulation of neurogenesis could have a retrograde effect after ischemia.
Middle cerebral artery oclussion (MCAO) was performed by the ligature model C57BL/6J mice. To check hippocampal-dependent tasks, contextual fear conditioning (CFC) and the Barnes maze were used. To increase neurogenesis, we used voluntary running. To suppress neurogenesis, we used temozolomide (TMZ; 25 mg/kg). Immunohistochemistry of Ki67, DCX, BrdU and NeuN were used to evaluate neurogenesis. Z-stack images were acquired with LSM-710-Confocal microscope.
We found that stroke increased hippocampal neurogenesis. In addition, ischemia also produced long-term hippocampal deficits with a decreased memory persistence. Next, we found that increasing neurogenesis by running increased neurogenesis, reduced freezing in the CFC and impaired performance in the Barnes-maze. On the contrary, reducing neurogenesis by TMZ improved CFC retrieval in ischemic mice.
Experimental interventions directed to manipulate neurogenesis after the formation of a post-stroke memory have a retrograde effect.

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C138
NOVEL COUMARINS AS INHIBITORS OF ENZYMATIC SYSTEMS INVOLVED IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is one of the most frequent multifactorial neurodegenerative disorders in elderly. In recent years, great advances have been made in the ability to design and test new synthetic molecules directed to one or several targets related to this pathology. Coumarins represent an important class of natural and/or synthetic oxygenated heterocycles showing a myriad of biological activities. Therefore, the current communication provides an overview about the potential of different substituted coumarins as anti-oxidant agents and inhibitors of enzymatic systems (MAO and BACE1) involved in AD.
The anti-oxidant activity of these compounds was studied in a scavenging activity of DPPH radical assay. MAO activity was determined by measuring their effects on the production of H_2O_2 from p-tyramine using the Amplex Red MAO assay kit and microsomal hMAO isoforms. BACE1 activity was evaluated by calculating the TR-FRET ratio using the LanthaScreen TR-FRET BACE1 assay kit. The theoretical evaluation of ADME-related physicochemical/structural parameters was performed using MolInspiration.
Many of the studied coumarins exhibited interesting activity and selectivity against hMAO-B isoenzyme, being some of them BACE1 inhibitors and promising anti-oxidant agents. Additionally, these molecules seem to present the desired theoretical ADME properties.
Our efforts are now focused in developing suitable chemical modifications to optimize the potential of these scaffolds as multi-target agents.

GASTROINTESTINAL-RESPIRATORY PHARMACOLOGY

C011
PHARMACOLOGICAL ACTIVATION OF WNT SIGNALLING INCREASE MUCOSAL HEALING IN STAT6 KNOCKOUT MICE

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Regeneration of the intestinal mucosa is a main goal in IBD-treatment. M2 macrophages mediate regenerative responses to injury and they have been related to Wnt ligands expression. STAT6$^{-/-}$ mice had an impaired M2 polarization and wound healing and we hypothesize that diminished mucosal Wnt signalling is associated with the delayed recovery observed in these mice.

WT or STAT6$^{-/-}$ mice received TNBS (3.5 mg/20 g mice, intrarectally) or vehicle (EtOH 40%) and were weighted daily. Colon length, mucosal histology and β-catenin protein expression were determined after sacrificing, 4 days after TNBS treatment. Some mice were treated daily with a Wnt agonist (5 mg/kg, i.p.) or its vehicle (5% DMSO).

The functional recovery of WT mice after TNBs administration was associated with increased β-catenin expression [immunostaining and nuclear protein levels (1.8 ± 0.1)] and increased mRNA expression of both c-myc (1.7 ± 0.1) and Lgr5 (2.8 ± 0.7) in the colonic mucosa. STAT6 (−/−) mice exhibited a delayed recovery and no significant changes in β-catenin protein levels and Lgr5 and c-myc mRNA expression was detected in the mucosa of TNBS-treated mice. In STAT6-TNBS mice, administration of a Wnt agonist compared with vehicle: (i) accelerated the recovery of body weight (92.6 ± 1.0% vs. 85.2 ± 1.3%), (ii) increased colon length (5.5 ± 0.1 vs. 4.6 ± 0.1 cm), (iii) reduced damage score (4.6 ± 0.4 vs. 6.5 ± 0.6) and (iv) augmented nuclear β-catenin protein levels (1.8 ± 0.2 vs. 1.0 ± 0.2), respectively.

A decreased Wnt signaling and delayed mucosal healing is observed in TNBS-treated STAT6 (−/−) mice. Systemic administration of a Wnt agonist increased intestinal Wnt signaling, mucosal regeneration and functional recovery of mice.

C013
THE FLESH ETHANOLIC EXTRACT (EH) OF *HYLOCEREUS* SP. EXERTS AN ANTI-INFLAMMATORY EFFECT AND PREVENT MURINE COLITIS

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IBD is a chronic disorder of the gastrointestinal tract characterized by disruption of epithelial barrier function and gut inflammation. Biologic therapies have significantly improved the course of the disease but there are still a high percentage of patients that do not respond to current therapies. We aim to evaluate the effects of the flesh ethanolic extract of *Hylocerus* sp. (EH) in a murine model of colitis induced by TNBS.

Balb/c mice received TNBS (3.5 mg/20 g, intrarectally) or vehicle (EtOH 40%) and 6 h later were administered with EH (20 mg/20 g mice, i.p.) or its vehicle (DMEM). Mice were weighted daily and after sacrificing (2 days after TNBS) we analyzed mucosal histology, Myeloperoxidase activity (MPO), the expression of pro-inflammatory molecules (qPCR) and protein levels of NF-κB and Iκβ-α.

The administration of EH compared with vehicle prevented ($P < 0.05$) the loss of body weight (91.9 ± 1.7% vs. 86.4 ± 2.1%) and significantly reduced: (i) histological damage score (3.6 ± 0.6 vs. 7.0 ± 1.0), (ii) MPO activity (1.3 ± 0.2 vs. 8.8 ± 0.3 U) (iii) the expression of pro-inflammatory molecules (Table 1) and (iv) Iκβ-α degradation and nuclear NF-κβ translocation in the colon.

Table 1. mRNA expression of different molecules detected in the colon of TNBS-treated mice. Results are expressed as fold induction vs. the value obtained in mice t hat did not receive TNBS

Systemic administration of the ethanolic extract of Hylocereus sp exerts an anti-inflammatory effect and prevents murine colitis induced by TNBS.

C036
PLASMABLAST RESPONSES INDUCED BY ORAL ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC) VACCINATION

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Enterotoxigenic *E. coli* (ETEC) is the most frequent bacterial cause of diarrhea in children in developing countries and the major cause of travelers' diarrhea; however, no ETEC vaccine is yet available. The development of new oral vaccines against ETEC and other enteric infections is hampered by the lack of suitable methods for analysis of intestinal immune responses in large scale trials or studies in children, the evaluation of blood plasmablasts and its trafficking receptor expression might provide an efficient overview about B cell response to enteric vaccines at the mucosal surface. Objectives: In this study, we established a flow cytometry assay to study plasmablast responses induced by ETEC vaccination using cryopreserved peripheral blood mononuclear cells (PBMCs).

PBMCs were isolated from healthy Swedish adults before and after receiving an oral multivalent ETEC vaccine (MEV). Plasmablasts were detected in cryopreserved cell samples using multicolor flow cytometry.

Significantly increased frequencies of circulating plasmablasts were detected in post compared to prevaccination samples. Plasmablast responses were primarily observed in subjects classified as responders, but the expression of the gut homing marker integrin β7 was significantly augmented among plasmablasts in both responders and weak/non responders after vaccination. Plasmablasts expressed IgA correlated significantly with the magnitudes of specific IgA responses primary vaccine antigens.

Our results demonstrate that plasmablast responses can be used as a quick test to evaluate the immunogenicity of oral vaccines as well as a tool to further characterize the phenotype and function gut homing plasmablasts.

C066
THE ANTIDEPRESSANT AGOMELATINE REDUCES INTESTINAL INFLAMMATION IN DNBS EXPERIMENTAL COLITIS IN MICE

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Intestinal inflammatory conditions frequently interact with depression and anxiety, having mood and stress a great impact in digestive disorders. Agomelatine is a novel well-tolerated antidepressant that combines melatonin agonistic and 5-HT2 antagonistic properties, both of great interest for the treatment of intestinal inflammation and the stress associated with this process. Therefore, we aimed to evaluate the effect of agomelatine in the DNBS model of colitis.
Acute intestinal inflammation was induced by intrarectal administration of DNBS (4 mg) in 50% ethanol to CD1 mice anesthetized with isoflurane. After 6 days of treatment with oral Agomelatine (50, 25 and 12.5 mg/kg), all groups were sacrificed and the colonic specimens were evaluated by determining the expression of proinflammatory markers and micro-RNAs by qRT-PCR.
The administration of agomelatine showed intestine anti-inflammatory effects as evidenced by a reduction in weight loss and mortality in the colitic groups treated with the doses of 50 and 25 mg/kg. Biochemically, this beneficial effect was evidenced by reduced expression of different proinflammatory markers, including cytokines and chemokines, metalloproteinases and barrier function and leukocyte adhesion molecules; additionally, some of the microRNA evaluated were modified, achieving a significant reduction in miR-9 and miR-223.
Agomelatine showed a direct intestinal anti-inflammatory effect, increasing the survival to an acute intestinal inflammation and affecting both the immune response and epithelial barrier function. Modifications achieved in some of the microRNAs could be involved in the mechanism of agomelatine directly reducing the inflammatory response.

C098
INDOMETHACIN INDUCES A DIFFERENTIAL REGULATION OF AUTOPHAGY IN JEJUNUM AND ILEUM

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NSAID-induced gastroduodenal toxicity has been thoroughly studied, but these drugs also cause damage in more distal parts of the small intestine and the mechanisms responsible for this enteropathy are largely unknown. Autophagy is a catabolic process that degrades superfluous and damaged organelles and represents a defensive mechanism that allows cells to survive under challenging circumstances. Our aim is to study whether the indomethacin-induced enteropathy associates with changes in intestinal autophagy.
BALB/C mice were treated with 10 mg/kg indomethacin (sc.) for 24 h. The presence of ulcers along the small intestine was assessed histologically and the intestinal autophagic flux was studied in samples of jejunum and ileum by measuring the levels of an autophagosome component (LC3-II protein) and the accumulation of an autophagic substrate (p62 protein).
The histological analysis revealed that indomethacin caused mucosal lesions in the ileum, but not in the jejunum. Western blot analysis showed that indomethacin treatment increased LC3-II protein levels and decreased p62 protein levels in jejunum, indicating an activation of autophagy. In contrast, both LC3-II and p62 protein levels accumulated in ileal samples, which suggest that autophagy is inhibited in this territory.
Autophagy is activated in the jejunum and inhibited in the ileum of mice treated with an enterotoxic dose of indomethacin, which suggest that autophagy constitutes a defensive mechanism that preserves mucosal integrity in the jejunum while its deterioration makes the ileum more susceptible to indomethacin-induced damage.

C099
MODULATION OF AUTOPHAGY REGULATES ASPIRIN-INDUCED GASTRIC DAMAGE IN A RAT MODEL

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Gastrointestinal damage is the major concern regarding treatment with non-steroideal anti-inflammatory drugs (NSAIDs). Autophagy is an intracellular process for degradation of long-lived proteins and organelles, which contributes to basal cellular homeostasia and acts as a survival mechanism under conditions of stress. We have previously shown that a single dose of aspirin reduces autophagy in the gastric epithelium of rats. We aim to study the effect of autophagy inhibition or stimulation on the extension of gastric damage induced by aspirin.
Male Sprague-Dawley rats were treated with aspirin (150 mg/kg, p.o.) for 3 h. F or autophagy inhibition we pre-treated some rats with 3-methyladenine (30 mg/kg, i.p., 45 min before aspirin) and for autophagy stimulation we treated some rats with rapamycin (1 mg/kg, i.p., with aspirin). After aspirin treatment rats were sacrificed and their stomachs were removed, opened along the greater curvature, pinned to a wax block, and photographed using a digital camera. Damaged area in the corpus region was quantified using the Image j software.
Gastric mucosa of rats treated with aspirin presented multiple haemorragic wounds ($1.31 \pm 0.27\%$ of total area). Rats receiving 3-methyladenine had a significant increase in the percentage of damaged area in the gastric corpus ($4.10 \pm 1.74\%$), whereas those rats treated with rapamycin were protected from damage ($0.51 \pm 0.28\%$).
Modulation of autophagy in the gastric mucosa affects aspirin-induced damage levels. Pharmacological activation of autophagy in t he stomach could be protective against gastric damage induced by aspirin or other NSAIDs.

C100
INDOMETHACIN INHIBITS AUTOPHAGY IN GASTROINTESTINAL EPITHELIAL CELLS

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NSAIDs have demonstrated chemopreventive activity against gastrointestinal cancers but their toxicity limits their use as prophylactics. They have now been postulated as possible adjuvants to surgery or chemotherapy in order to prevent the formation of new lesions and reduce disease progression. Cancer cells enhance their resistance to the cytotoxic action of antineoplastic drugs by activating autophagy, which is a catabolic process that degrades superfluous and damaged organelles in order to escape death. Our aim is to analyze whether indomethacin modulates autophagy in gastric and colon cancer cells.
Gastric AGS cells and colonic HT-29 cells were treated with increasing concentrations of indomethacin for 24 h and the autophagic flux

was evaluated by measuring LC3-II and p62 protein levels (Western blot) in control conditions and in the presence of different lysosomal inhibitors (choroloquine, ammonium chloride and bafilomycin-B1). In some cases, the effects of indomethacin were evaluated in cells treated with activators (rapamycin) or inhibitors (wortmanin) of autophagy.
Treatment of both cell types with indomethacin increased LC3-II and p62 basal levels but did not enlarge the accumulation provoked by lysosomal inhibitors, which suggests that indomethacin inhibits the basal autophagic process at a late step. Accordingly, indomethacin inhibited the autophagic degradation of p62 induced by rapamycin while the early-acting autophagy inhibitor wortmanin reduced the accumulation of LC3-II provoked by indomethacin.
Indomethacin inhibits autophagy in gastric and colonic cancer cells by acting at a late stage in this catabolic process, an effect that probably renders them more susceptible to cytotoxic drugs.

C114
CHARACTERIZATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN AUTOPHAGY-RELATED GENES IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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Inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease (CD), is a highly prevalent pathology that involves chronic inflammation of the digestive tract. During the last years, some single nucleotide polymorphisms (SNP) associated with genes in the autophagy pathway have been identified as involved in susceptibility to IBD. Our objective is to analyse the presence of different SNPs in autophagy-related genes in patients with IBD in order to determine alterations in the evolution of the disease and the response to treatment.
We studied 43 patients diagnosed with IBD (30 with CD and 13 with UC) in the Hospital of Manises. DNA was extracted from blood samples and SNPs in ATG16L1, NOD2, IRGM, PTPN2 and LRRK2 genes were genotyped using PCR-RFLP. Also, we collected pathological characteristics of each patient.
SNP genotyping showed that the number of risk alleles is higher in CD than in UC patients (1.700 ± 0.145 vs. 1.000 ± 0.160, $P < 0.01$). In CD subjects there is no correlation between the presence of risk alleles and the age at diagnosis, location nor behavior. However, the greater is the number of risk alleles in CD patients, the lower is the time elapsed from diagnosis to the start with the immunosuppressive therapy ($r = 0.3761$, $P = 0.0532$).
The analysis of the presence of risk alleles in SNP in autophagy-related genes may be effective to investigate the importance of autophagy in the evolution and treatment of IBD patients.

C124
NOVEL PROTECTIVE EFFECT OF AN INDOL DERIVATE ON DSS-INDUCED ACUTE COLITIS IN BALB/C MICE

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UC is a chronic disease of the gastrointestinal tract characterized by relapses of severe intestinal inflammation and impacts significantly on quality of life. So far, no cure has been found and further treatment options are required to prevent severe complications. To reduce intestinal inflammation in acute relapses, anti-inflammatory drugs are an integral part of the current therapy. The indol derivate 5-bromo-1-ethyl-3-[(E)-4-(furan-2-yl)-2-oxobut-3-enyl]-3-hydroxyindol-2-one (RZ) has already shown to be an effective Akt and β-catenin inhibitor and demonstrated activity over both a colorectal and a prostate cancer cell lines (Zanni et al., 2015). The aim of this study was to assess whether RZ can inhibit the development of acute colitis induced by DSS in Balb/C mice.
Acute colitis was induced to Balb/C mice through oral administration of 3% DSS (w/v) in water for 7 days. Animals were randomly assigned to four groups: Blank, Blank + RZ (70 mg/kg, 0.2 ml by gavage), DSS and DSS + RZ (70 mg/kg, 0.2 ml by gavage). After animals were sacrificed at day 8, mice colon were weighted and the length measured, cut in small pieces of 0.5 cm, and submitted to histological, biochemical and molecular analysis.
Treatment with RZ improved DAI score, protected from colon shortening ($P < 0.01$), and reduced intestinal neutrophil infiltration ($P < 0.01$). Moreover, RZ-treated mice showed a decrease in IL-6 tissue levels, COX-2 ($P < 0.01$) and iNOS ($P < 0.01$) expression. Analysis of NF-κB pathway, demonstrated a clear inhibition of p65 nuclear translocation by RZ ($P < 0.01$).
RZ seems to be a potential therapeutic agent against ulcerative colitis.

HORMONAL PHARMACOLOGY AND METABOLISM

C012
INVOLVEMENT OF AROMATASE P450 IN THE MODULATION OF PITUITARY LH-POSITIVE CELLS IN MICE

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For knowing the relevance of aromatase P450 in maintaining the pituitary function and its pharmacological implications, mainly associated with reproduction, a study of LH-positive cells in the pituitary of female and male mice knocked-out (KO) was performed for this enzyme.
Pituitary glands obtained from WT and aromatase P450 KO mice of both gender underwent immunohistochemical staining followed by morphometric and planimetric analysis.
In the KO animals, a significant increase in the cellular, nuclear and cytoplasmic areas was observed in the LH-positive cells, after comparing with the WT mice. Furthermore, LH-positive cells were more abundant in the KO than in the WT group. In all regions of the gland, LH-positive cells in KO mice were characterized by a higher intensity of cytoplasmic reaction than in WT. Overall, LH-positive cells were more polygonal and exhibited more short and thick cytoplasmic prolongations in KO than in the WT mice. Besides, the LH-positive cells showed a greater proliferative rate in the KO mice. Altogether, the findings observed are signs of morphological cellular hyperactivity, associated with an increase in the size of the cell and nuclear areas and an increment in the percentage of LH-positive cells.
Because similar outcomes were obtained in male and female KO mice, our findings suggest that the local production of estradiol is necessary for the regulation of LH-gonadotropic cells, by modulating pituitary population of LH-positive cells in males (and perhaps in females), which could explain the higher pituitary enzyme expression in the male mice.

C033
EFFECT OF GLYCOLYSIS INHIBITION IN THE KINETICS AND OXIDATIVE HOMEOSTASIS OF RAM SPERM

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Spermatozoa are extremely susceptible to oxidative stress (OS). In fact, OS has been referred as the main cause of male infertility. Studies have suggested that mitochondria are the main source of energy for sperm metabolism; however, they are also the main source of reactive oxygen species (ROS). Nevertheless, recent studies have demonstrated that glycolysis also participate on sperm physiology. The aim of this study was to evaluate the role of glycolysis on sperm motility and oxidative status. Semen samples of 10 rams were diluted at a concentration of 200×10^6 sperm/ml and divided into four treatments: control group and three concentrations of the glycolysis inhibitor 2-Deoxy-D-glucose (DOG; 5, 10 and 50 mM). Samples were then incubated at 37°C for 30 min, and subsequently assessed for computer assisted sperm analysis, mitochondrial membrane potential (MMP; fluorescent probe JC-1) and lipid peroxidation (TBARS assay). Samples treated with 50 mM DOG had a lower percentage of cells with low MMP compared to the control group, suggesting a mitochondrial compensation to supply glycolysis inhibition. Furthermore we observed a concentration-dependent increase on lipid peroxidation probably due to deprivation of pyruvate from the glycolytic pathway that may have induced mitochondrial dysfunction. In addition, we observed in all treated groups, changes in the patterns of sperm movement. These results indicate that glycolysis may be an important source of ATP for sperm kinetics and that inhibition of glycolytic route may indirectly lead to sperm oxidative stress. These data may provide clues for the pharmacological antioxidant therapy for strategic treatment against infertility.

C080
EFFECTS OF N- AND C-TERMINAL PARATHYROID HORMONE-RELATED PROTEINS ON SENESCENT OSTEOARTHRITIC HUMAN OSTEOBLATS

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Premature cellular senescence is known to occur in chronic inflammatory diseases including osteoarthritis (OA). Parathyroid hormone-related protein (PTHrP) might be considered a promising bone anabolic therapy. We have investigated the ability of PTHrP (1–37), PTHrP (107–139) and PTHrP (107–111; osteostatin) in the regulation of stress-induced senescence occurring in OA osteoblasts stimulated with interleukin-1β (IL-1β).
Osteoblasts were obtained from patients undergoing total knee joint replacement. Subchondral bone tissue was minced and digested with collagenase, and seeded in osteogenic medium to obtain osteoblastic cells according to a standard procedure. At first passage, osteoblastic cells were treated with PTHrP (1–37), PTHrP (107–139) and osteostatin (each at 100 nM) with or without IL-1β (10 ng/ml) for 1, 3 and 7 days. Cells were characterized by flow cytometry and mineralization with red alizarin. Senescence-associated β-galactosidase activity was measured by immunocytochemistry. mRNA expression of caveolin, p53 and p21 by PCR; detection of H2AX foci and actin cytoskeleton by immunofluorescence.
Cells were positive for osteoblast markers CD105 and CD90, and negative for CD45 and CD34. β-galactosidase activity and senescence-associated to heterochromatic foci are increased by IL-1β, but this effect is decreased by the action of the peptides, mainly with PTHrP (107–139) at 7 days. PTHrP maintained the normal phenotype of actin filaments and increased mineralization at longer times. Also, PTHrP peptides prevented the upregulation of caveolin-1, p53 and p21 induced by IL-1β.
These findings show that both N- and C-terminal PTHrP peptides may contribute to the down-regulation of important senescence markers in OA osteoblasts.

C102
NAMPT AS KEY TARGET ON DIETARY FATS-INDUCED POSTPRANDIAL INFLAMMATION IN METABOLIC SYNDROME

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The metabolic syndrome is a state of chronic low-grade inflammation as a consequence of complex interplay between genetic and environmental factors. Several adipokines such IL-6, and NAMPT are overexpressed during fat abdominal accumulation, which later will lead to obesity disorders. Herein, we have sought to assess whether olive oil (MUFA), compared to other dietary fatty acids and in association with vitamin B3 could have benefits on NAMPT-related postprandial inflammation by means of monocytes activation and pro-inflammatory cytokines modulation as IL-6 and TNFα. Monocyte activation was assessed by flow cytometry and gene expression analysis of different specific markers, as well as proinflamatory cytokines. On the other hand, ex vivo studies with human primary monocytes were performed. These monocytes were pretreated with FK866, Lentivirus iNAMPT and eNAMPT treatment before the exposure to SFA-, MUFA- and PUFA-TRL in absence or presence of vitamin B3. IL-6 mRNA levels and secreted protein were determined by RT-PCR and ELISA respectively. It was interesting to note that the ingestion of SFA-enriched meal induced a postprandial increase in the number of classical monocytes, moreover, IL-6 and TNFα gene expression was upregulated in postprandial serum and monocytes. Isolated human monocytes treat with postprandial SFA-TRL expressed highly IL-6, but was reduced in monocytes co-treated with vitamin B3. The presence of FK866, NAMPT inhibitor, provoked a dramatically IL-6 release from monocytes. NAMPT is shown to have synergy acting with MUFA and MUFA-ω3 with a compromised mobilization of pro-inflammatory monocyte, which improves the postprandial pro-inflammatory state triggered by fatty acid-enriched meals.

C122
IDENTIFICATION OF BETATROPHIN IN FAT TISSUE AND DIFFERENCES ON ITS LEVELS RELATED TO SEX IN OBESE PATIENTS

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Fat tissue secretes several peptides (also called adipokines) some of which are directly related to adipose tissue dysfunction in obesity, increasing the risk of metabolic and cardiovascular diseases. The identification of betatrophin, which augments dramatically the proliferation of pancreatic beta-cell in insulin resistant mice, has awakened great expectations, but the results in humans are contradictory. The aim of this study was to analyze the role of betatrophin in control and obese patients, correlating with others adipokines, and its expression in fat tissue, to assess whether the regulation of this protein could be a new therapeutic approach in the prevention and/or treatment for obesity and DM2.

Thirty-six patients obese and controls, were recruited at surgery service with informed consent. The plasma levels of betatrophin and adiponectin were measured by ELISA. In isolated adipocytes from the visceral adipose tissue, lipolysis was determined by glycerol release. To analyze the presence of betatrophin in fat tissue western blotting and immunocytochemistry were performed.

The lipolytic effect on obese is clearly damaged vs. non-obese. Betatrophin levels oscillated from 0.17 to 4.63 ng/ml, being higher in obese women than men ($P < 0.01$) and similar results were described to adiponectin ($P < 0.01$). In adipose tissue from obese, betatrophin was detected by immunocytochemistry, however the western blot showed only a weak signal.

These results confirm the presence of betatrophin in plasma and the immunocytochemistry suggests that this bioactive peptide is present in fat tissue. Besides, adiponectin and betatrophin show sexual dimorphism in obese patients.

MOLECULAR PHARMACOLOGY

C014
CXCR6/CXCL16 AXIS IS INVOLVED IN ABDOMINAL AORTIC ANEURYSM (AAA) FORMATION INDUCED BY ANGIOTENSIN II

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Abdominal aortic aneurysm (AAA) is a degenerative disease of the aorta that mainly affects elderly population over the age of 65. Nowadays the pathways involved in its onset and progression remain unknown and angiotensin-II (Ang-II) has been widely implicated. Therefore, the potential link between CXCR6/CXCL16 axis in AAA was investigated.

Apolipoprotein E-deficient mice (apoE$^{-/-}$) were subjected or not to a high-fat diet and infused with Ang-II (500 ng/kg/min) for 28 days. Some of the animals were daily treated with losartan at 10 or 30 mg/kg/day. Parallel-plate flow chamber assay was employed to evaluate leukocyte adhesion to Ang-II (1 μM)-stimulated human endothelium.

Mice subjected to a high-fat diet and infused with Ang-II showed higher incidence of AAA, increased macrophage, CD3$^+$ lymphocyte and CXCR6$^+$ cell infiltration and enhanced neovascularization than unchallenged animals. These effects were accompanied by increased MCP-1/CCL2, CXCL16, CXCR6 and VEGF mRNA expression within the lesion. These events were reduced when losartan was administered at 30 but not at 10 mg/kg/day. When human umbilical vein or artery endothelial cells (HUVEC and HUAEC, respectively) were stimulated with 1 μM Ang-II (24 h), a significant increase in CXCL16 expression was detected by flow cytometry and immunofluorescence. However, neutralization of CXCL16 activity only significantly inhibited Ang-II-induced mononuclear leukocyte-HUAEC interaction by 49% without affecting their interaction with HUVEC.

These results suggest that the CXCR6/CXCL16 axis could constitute a new therapeutic strategy in the treatment of cardiovascular diseases associated with activation of the renin-angiotensin system (RAS).

C050
MODERATE HEAT STRESS IN HEPATIC CELLS MEDIATES TEMPORAL DYNAMICS OF CRUCIAL CELL SURVIVAL FACTORS

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Heat preconditioning is an adaptive mechanism that protects cells from different kinds of injury. It is mediated by the induction of heat shock proteins (HSPs), being the intensity of heat stress (HS) and the duration of subsequent recovery vital parameters.

This study evaluates the effects of moderate HS (43°C/45 min) and time-dependent changes during the recovery period of the gene and protein expression of crucial cell survival factors: HSP70, Bcl-2 and p53 in HepG2 cells. We also evaluated the effects of 0.4 mM aspirin, a pharmacological HSP co-inducer, alone (ASA) and in a combination with HS (ASA + HS).

HS alone and ASA + HS caused a major up-regulation of HSP70 mRNA in the first 2 h, while HSP70 protein increased gradually and was especially abundant 2–24 h. Regarding Bcl-2, all treatments rendered similar results: gene expression was down-regulated in the first 2 h, after which there was protein elevation (12–48 h after HS). P53 mRNA expression was down-regulated in the first 12 h in both HS- and ASA + HS-cells. The immediate decrease of p53 protein after HS was followed by a biphasic increase.

HepG2 cells are most vulnerable in the first 2–6 h, but are endowed with capacity for combating stress at 12–24 h after HS. In this model, ASA does not act as a co-inducer of HSP70, but promotes Bcl-2 protein expression with prolonged treatment. Finally, short term exposure to moderate heat may be a 'physiological conditioner' for liver cells to accumulate HSP and Bcl-2 and thus obtain cytoprotection.

C079
IDENTIFICATION OF NOVEL MOLECULES TARGETING CARTILAGE AGING AS OSTEOARTHRITIS THERAPEUTICS

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Effective treatments for osteoarthritis (OA) are not available. In aging-related diseases, including OA, failure of cellular homeostasis mechanisms, such as, autophagy can cause extracellular matrix destruction and cell death. Chondrocytes are required for maintenance of cartilage integrity. With aging, chondrocyte function is limited, contributing to a cellular senescence phenotype often observed in OA chondrocytes. The objective of this study was to identify dual anti-senescence and pro-autophagy molecules by a cell-based high content imaging.

Human chondrocytes were seeded (3000 cells/well) in 384 well plates, treated with IL-6 (20 ng/ml) to induce cellular senescence and defective autophagy and incubated with a subset of BioFarma Chemical Library (3000 compounds) at 10 μM for 72 h for the senescence phenotype and for 18 h for the autophagy phenotype. Imagene Green C12FDG and Cyto-ID® Autophagy detection kit were employed to measure senescence-associated β-galactosidase activity and to monitor autophagy flux, respectively. High content cellular imaging and analysis Operetta system was employed to determine fluorescence labeled β–galactosidase subcellular structures and autophagy vacuoles.

A set of compounds with anti-senescence effects was identified by senescence-associated β-galactosidase activity screen. Anti-senescence compounds were screened for autophagy flux and a subset of compounds with pro-autophagy effects was selected. Further studies are necessary to validate the dual anti-senescence and pro-autophagy active compounds as chondroprotective agents.

The chemical tools identified provide a unique opportunity to target cartilage aging key events (chondrocyte senescence and autophagy) as a therapeutic strategy to protect from aging-associated changes and prevent the onset of OA.

C084
A NOVEL CELL LINE FOR STUDYING DOPAMINE D2 AND SEROTONIN 5-HT2A HETERODIMERIZATION

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It has been shown that serotonin 5-HT2A (5-HT2AR) and dopamine D2 receptors (D2R) are expressed as heterodimers. This heterodimerization has been reported as a key for the antipsychotic drugs mechanism of action and it modifies the ligand efficiency when compared with isolated receptors.

Our aim was the development and characterization of a double-stable HEK293 cell line expressing human D2R and 5-HT2AR, useful for gaining knowledge about the role of heterodimerization in these receptors' pharmacology.

We generated a double-stable Flp-In HEK293 cell line expressing D2R tagged with YFP and able to express 5-HT2AR tagged with CFP, in a doxycycline inducible manner. The optimal conditions (10 ng/ml doxycycline for 24 h) to induce the expression of the 5-HT2AR were established by fluorescence microscopy.

The pharmacology was evaluated by means of membrane radioligand binding studies. D2R were labelled with [3H]spiperone (KD = 1.18 nM) and 5-HT2AR with [3H]ketanserin (KD = 1.63). Binding competition with haloperidol and risperidone at D2R showed Ki values of 4.92 nM and 13 nM and at 5-HT2AR showed Ki values of 80.42 nM and 0.96 nM, respectively.

We also evaluated the functional properties of both receptors by measuring inhibition of cAMP when D2R were activated by dopamine (EC_{50} = 30.74 ± 17.56 nM) and accumulation of inositol phosphates when 5-HT2AR were activated by 5-HT (EC_{50} = 151.63 ± 107.44 nM).

The developed cell line expressing D2R permanently and 5-HT2AR in an inducible manner constitutes a novel tool for studying functional and pharmacological consequences of D2R and 5-HT2AR heterodimerization and its regulation by antipsychotic drugs.

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C092
PREDICTION OF POTENTIAL ANTIOXIDANT DRUGS THROUGH CHEMOMETRIC TECHNIQUES

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Oxidative stress is associated with a variety of pathologies. Consequently, the research for new antioxidant molecules is increasing.

Aim: To predict antioxidant activity of target molecules by using molecular descriptors designed with a model based on Molecular Topology.

A group of 112 and 113 molecules with and without known antioxidant activity respectively were selected. 657 molecular descriptors obtained with PaDEL-Descriptor software were chosen to do linear discriminant analysis with the statistical program SPSS. 70% of the molecules were used to create the model and 30% to validate it. Molecules with a value in the discriminant function over 0 were considered antioxidants. The discriminant function obtained was used to search active principles in the 2013 Spanish Catalogue of Drugs.

The discriminant function obtained was: DF = 28.545 + 0.196C3SP2 + 0.402nHBd + 0.136nHdsCH + 0.115nsCH3 + 5.589nssSe + 2.706minHsOH – 2.891minHssNH + 5.784 min dS – 58.288ETA_EPSILON_3 – 10.584ETA_Psi_1 − 0.202nHBAcc- + 1.364topo Shape. This function allows the successful classification of the training molecules into active and non-active. The global cross validation was 93.64%. When the function was applied to the active test group the success obtained was 95.24% and 92% when applied to the inactive one. 77 potential antioxidant drugs has been detected in the 2013 Spanish Catalogue of Drugs.

The use of chemometric methods like linear discriminant analysis is a successful tool to predict the antioxidant activity of drugs.

C117
EMERGENCE, SPREAD AND UNCONTROLLED EBOLA OUTBREAK

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It is interesting to determine the factors contributing to the emergence, rapid spread and nature of 2014 virus outbreak. Nearly 60% of all human pathogens are of zoonotic origin, e.g., those that have only recently jumped to humans [Ebola virus disease (EVD) identified in 1976]. A pathogen spread relies on perpetual contact with new groups of susceptible individuals. Ideal conditions for zoonotic-viruses emergence and spread are socioeconomic and environmental changes, long-distance mobility and changing climate. Akhtar et al. reviewed the emergence of EVD outbreak. In the present report, Akhtar et al.'s model of EVD is analyzed with the aim to provide a broad sketch of the fundamental human–Ebola-virus biophysical forces that enable and constrain EVD.

Akhtar et al. proposed a model of Ebola virus transmission dynamics and emergence of EVD outbreak.

With Akhtar et al.'s model, the emergence of EVD outbreak was analyzed. Cells of the mononuclear phagocyte system are the first ones to be manipulated by the virus, and responsible for providing the host with innate and adaptive immunity. The model explains how Ebola evades the immune system, providing a broad sketch of the fundamental human–Ebola-virus biophysical forces that enable and constrain EVD.

We are not prepared for viral outbreaks: (i) state of knowledge about host-pathogen interactions is selective; (ii) deficit of trained medical and scientific personnel delays deployment to the established Ebola treatment centres in West Africa; (iii) public is unaware of the threat of emerging viral diseases.

CLINICAL PHARMACOLOGY, SIDE EFFECTS AND TOXICOLOGY

C021
LIVER INJURY DUE TO HERBALS AND DIETARY SUPPLEMENTS: A CAUSE FOR CONCERN

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Heterogeneous regulatory framework of herbals and dietary supplements (HDS), their widespread use and unawareness of health risks, particularly liver injury, are main causes for concern. We aim to evaluate clinical phenotype and outcome associated with HDS hepatotoxicity (DILI).

Demographics and clinical-biochemical parameters were compared between HDS and conventional medication DILI cases included in the Spanish and Latin-American DILI registries.

Out of 1025 DILI cases included, 26 were induced by anabolic steroid (AAS) and 44 by herbals and other dietary supplements (HDS). Women (60%) predominated among the Spanish HDS-DILI cases. The HDS-DILI mean age was significantly higher than that of AAS-DILI cases, but lower for conventional drugs (48 vs. 33 and 54; $P < 0.001$) among the Spanish and similarly the Latin-American cases.

The Spanish HDS cases presented higher ALT mean value (39× ULN) compared to AAS (15× ULN) and conventional medications (20× ULN; $P < 0.001$) respectively, but lower total bilirubin values than AAS-DILI cases (9 vs. 17× ULN; $P < 0.001$). Similar results were found among the Latin-American cases.

In total, HDS-DILI patients developed acute liver failure (ALF) more frequently than DILI patients associated with conventional drugs (9% vs. 5%; $P = 0.03$). No AAS cases developed ALF. Rechallenge was more frequent with HDS than with conventional medication in both registries (14% vs. 6%; $P = 0.02$).

In comparison to conventional drugs, HDS-DILI is more common in young women, presenting with hepatocellular injury, higher transaminase values, higher risk of ALF and inadvertent re-exposition. Greater awareness and stricter regulation are required to prevent HDS-related severe adverse effects to the liver.

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C034
DOES CYCLOPHOSPHAMIDE TREATMENT CAUSE ACTIVATION OF RETINAL MICROGLIA?

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Cyclophosphamide (CPA) is an immunosuppressive agent that is effective in the treatment of a large number of autoimmune diseases and vasculitis syndromes, to improve ocular manifestations and to maintain visual acuity in patients with immune system-related diseases. Although ocular toxicity of CPA has not been reported, our purpose was to study whether CPA treatment might cause activation of murine retinal microglia, as excessive or prolonged microglia activation could become deleterious for patients with retinal neurodegenerative diseases (RND). Activation of retinal microglia after CPA treatment was determined by flow cytometry (measured as increased forward scatter heterogeneity of the CD11b positive population and increased expression levels of CD11b) and confirmed by confocal immunofluorescence analysis (cell morphology and immunoreactivity against anti-IBA1 and anti-MHCIIRT1B antibodies). CPA treatment caused activation of retinal microglia, which increased with concomitant peripheral inflammation (chemically induced sublethal colitis). In these conditions translocation of gut commensal bacteria to the liver was observed, as well as an increased expression of Toll like receptor 2 (TLR2) in activated retinal microglial cells. Likewise, in Candida albicans colonized mice, CPA treatment allowed fungal translocation from the gut to internal organs, and caused retinal microglia activation. These results suggest that microglia activation is probably mediated by recognition of microbial ligands through pattern recognition receptors, such as TLR2, and/or by proinflammatory-circulating cytokines. Therefore, immunosuppressive treatments may represent a risk factor for patients with RND (as diabetic retinopathy or glaucoma), due to their potential to activate retinal microglia.

C085
POTENTIALLY INAPPROPRIATE MEDICATION IN OLDER PATIENTS WHO ATTEND TO A COMMUNITY PHARMACY

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Drugs are classified as potentially inappropriate when the risk of adverse effects is superior to clinical benefit. Several criteria have been developed to improve correct prescription in the elderly. The aim of this study is to analyze the pharmacotherapy of elderly patients who attend to community pharmacies to detect potentially inappropriate prescriptions (STOPP criteria) and potentially omitted prescriptions (START criteria), determining their prevalence.

A descriptive-observational study was performed with 311 patients who attend to any of the two community pharmacies of Ondara (Spain) over 2 months. Patients had at least three chronic treatments and were older than 65 years. The suitability of the medication was verified according to the revised STOPP-START criteria (1).

The mean age of the patients was 76 years (SD = 7). The total prescribed medications were 2171 with an average of 7 medications per patient. According to STOPP criteria, 60.1% of the patients had at least one potentially inappropriate prescription. The most prevalent criterion was benzodiazepines for ≥4 weeks (32.5%). Using START criteria, 114 potentially omitted prescriptions (36.7% of the patients) were detected. The most prevalent criterion was laxatives in patients receiving opioids regularly (12.8%). A collaborative project with the primary care doctors is in progress.

Community pharmacists' review of medications, using STOPP-START criteria, may help to identify potentially inappropriate prescription and to detect possible drug related problems.

(1) Delgado-Silveira, E, et al. (2014). *Rev Esp Geriatr Gerontol* 50: 89–96.

C094
ADVERSE REACTIONS TO DRUGS ANTI-TUMOR NECROSIS FACTOR ALPHA: EVIDENCE IN REALCLINICAL PRACTICE

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The adverse reactions (ARs) of Anti-Tumor Necrosis Factor Alpha (Anti-TNFa) drug can be a clinical problem by compromising patient safety. The purpose of the study is to characterize the ARs Anti-TNFa in real-clinical practice.
Multicenter study (5 hospitals) national observational and retrospective with a time horizon of 5 years (June 2008–May 2013). Dispensations of patients >18 years diagnosed with rheumatoid arthritis and ankylosing spondylitis and at least 3 months in Anti-TNFa therapy were included. Anti-TNFa were: Infliximab (INF), Adalimumab (ADA), Etanercept (ETN), Golimumab (GOL), Certolizumab (CTZ). The pharmacological variables were drugs, treatment time and reason for change and/or suspension Anti-TNF and safety were number and severity of ARs. The variables were obtained from the records of dispensing and patient clinical reports.
A total of 1276 patients were included and 1674 patient dispensations. The prescribed drugs were: 36.91% ADA, 35.30% ETN, 20.54% INF, 5.49% GOL y 1.73% CTZ. A total of 374 ARs Anti-TNFa were observed (0.3 ARs/patient). 5.79% were severe ARS that caused hospital admission or increased hospital stay and 9.91% caused the change or discontinuation of treatment Anti-TNFa. The main ARs detected in real-clinical practice were: 35.63% infections, 21.16% dermatological, 9.28% malaise, 4.75% cardiovascular, 4.10% gastrointestinal, 3.02% uveitis, 2.59% haematological, others were unspecified intolerances.
About 24.34% of the dispensed Anti-TNFa produced adverse reactions in real-clinical practice, some were serious and result in hospitalization or increased hospital stay. It is necessary to establish corrective measures focused on monitoring to prevent serious adverse reactions to Anti-TNFa drugs.

C104
ROLE OF THE PURINERGIC SYSTEM IN THE VASCULAR PRO-INFLAMMATORY EFFECTS OF ABACAVIR

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Abacavir (ABC) was associated with adverse cardiovascular effects whose underlying mechanisms remain unclear. We demonstrated that ABC induces leukocyte-endothelial cell interactions (early feature of the atherosclerotic plaque), effects that were reproduced by another purine analogue (didanosine), but not by pyrimidine analogues (lamivudine, zidovudine and emtricitabine) or the acyclic nucleotide analogue tenofovir. Given chemical similarity of ABC to purinergic mediators (ATP, ADP and AMP), we assessed the role of ATP-receptors in the leukocyte accumulation induced by ABC and the cell type involved.
Human umbilical vein endothelial cells (HUVEC) and polymorphonuclear leukocytes (PMN) were treated in all cases with ABC (5 μg/ml, 4 h) and leukocyte-endothelium interactions were measured using a flow-chamber system. In some cases, both cell types were pre-treated with the ATP-receptor antagonists indicated in Figure 1. To determine the implication of each cell type, they were pretreated individually with the antagonists.
ABC induced a decrease in PMN rolling velocity and an increase in PMN rolling flux and adhesion to endothelial cells. These interactions were absent following pretreatment with Suramin or either of the two P2X7 antagonists but were maintained by A317491 (Figure 1). Individual incubation with the antagonists implicated both leukocytes and endothelium in the interference of ABC with the purinergic receptors, although the former cell type played a more prominent role.
Our results suggest an interaction of ABC with vascular purinergic signaling that involves the specific activation of P2X7 receptors on leukocytes and endothelial cells. This interaction may underlie effects of ABC on cardiovascular system.

C123
THE INFLUENCE OF DRUG PHYSICOCHEMICAL PROPERTIES ON DELAYED ONSET IN HEPATOTOXICITY: AN ANALYSIS OF THE COHORT INCLUDED IN THE SPANISH DILI REGISTRY

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Most patients with drug-induced liver injury (DILI) manifest liver injury while they are still on the drug treatments; however some manifest after the treatment is completed (delayed onset). Mechanism underlying the delayed onset is unknown. We aimed to identify drug properties and host factors which are associated with delayed onset.
680 Spanish DILI cases were classified into delayed onset (DO) vs. no delayed onset (NDO) according to the temporal relationship of the first DILI manifestation to the time of treatment cessation. Drug properties and ho st factors were compared in DO vs. NDO.
Among the 680 cases, 159 cases (22%) manifested DILI with 2–82 days' delay after the treatment cessation. 57% of DO cases and 13% of NDO were Amoxicillin-clavulanate (AMOX/CLAV) cases. After excluding the AMOX/CLAV cases, DO cases had shorter treatment duration (median: 11 vs. 45 days, $P < 0.0001$) and higher daily dosage (mean: 275 vs. 150 mg, $P = 0.005$). Drugs with hepatic metabolism <50% were more prevalent in DO cases (84% vs. 67%, $P = 0.003$) as well as drugs with un-metabolized excretion >50% (25% vs. 9%, $P = 0.0011$). Eosinophilia was more prevalent in DO cases (30% vs. 19%, $P = 0.036$), while positive autoantibody was more prevalent in NDO (25% vs. 11%, $P = 0.024$). NDO cases were more associated with chronic underlying diseases (82% vs. 60%, $P < 0.0001$). In a multivariate analysis, the absence of underlying diseases (OR: 3.2) and un-metabolized drugs excretion (OR: 3.6) were found as predictors for DO. These results were consistent even when including AMOX/CLAV cases.
Delayed onset was associated with drugs which have low hepatic metabolism, high daily doses, manifestations with more eosinophilia and less positive autoantibodies and less chronic underlying diseases.
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C131
ESTIMATION OF COCAINE ABUSE IN SOUTHEAST OF SPAIN BY WASTEWATER ANALYSIS

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The use of cocaine, one of the most potent and addictive illicit drugs, appears to be increasing with significant consequences for human health and social behavior. It has been determined that residues of illicit drugs and their metabolites that are excreted by humans, flow into and through wastewater treatment plants, so the aim of this study was to determine the concentrations of cocaine in samples of wastewater from a water treatment plant in the Region de Murcia, and to use these data to estimate the consumption patterns in the population.

Metabolites derived from human use of cocaine were measured by mass spectrometry in wastewater samples collected from an urban treatment plant of Region of Murcia. Drug concentrations, population size and water flow rate were used to estimate local cocaine consumption.

Cocaine consumption average of analyzed days was 845,224 g/day. The highest concentrations coincided with the weekend, with a consumption average of 1510,595 g/day. Knowing inhabitant served by the flow under study, we calculated that an average of 5631 g of cocaine were consumed per day and 1000 inhabitants during analyzed days.

Cocaine consumption levels obtained in Region of Murcia are higher than levels obtained in similar studies carried out in Europe. Population should make aware about the great problem that drugs abuse mean. Public administration should carry out more campaign against drug abuse in order to decrease this great problem.

ONCOLOGIC

C029
EVALUATION OF SYNTHETIC CHALCONES ON CELL VIABILITY OF HUMAN LEUKEMIA CELL LINES

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P-glycoprotein is a member of the ATP-binding cassette transporter family which is involved in the multidrug resistance of cancer cells to several anti-cancer drugs.
To synthesize and to determine the effects of selected chalcones on viability of human leukemia cell lines and in particular P-glycoprotein-overexpressing K-562/ADR cells.
Chalcones were synthesized by a Claisen–Schmidt condensation of 2-hydroxyacetophenones and benzaldehydes and their structures were determined by spectroscopic analyses. HL-60, U-937, MOLT-3, K-562 and K-562/ADR cells were grown in RPMI 1640 medium and cytotoxicity was analyzed by colorimetric MTT assay. Cell cycle phase distribution and reactive oxygen species were determined by flow cytometry. The evaluation of apoptosis was carried out by fluorescent microscopy, flow cytometry and DNA fragmentation. Caspase activity was determined using colorimetric substrates, processing of caspases and release of mitochondrial proteins by Western blot.
We evaluated the antiproliferative activity of seventeen synthetic chalcones against human leukemia cell lines and found that 2′-hydroxy-6′-benzyloxy-4-bromochalcone was the most potent, showing IC50 values of approximately 5 mM, including the multidrug resistant K-562/ADR. This compound induced apoptosis in a concentration- and time-dependent manner and blocked cell cycle progression at the S phase. Cell death was found to be associated with the release of mitochondrial pro-apoptotic proteins, the cleavage and activation of caspases and an increase of intracellular reactive oxygen species generation.
The selected chalcone effectively induces cell death in leukemia cells that overexpress P-glycoprotein and could be a potential candidate for developing novel anti-cancer agents.

C070
ADDING PERTUZUMAB IN NEOADJUVANT TREATMENT OF PATIENTS WITH HER2$^+$ BREAST CANCER IN SPAIN: A COST OFFSETS STUDY

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Pertuzumab (Perjeta®) has recently been approved in Europe as part of the neoadjuvant treatment for patients with HER2-positive, locally advanced, inflammatory or early stage breast cancer (BC). We aimed to calculate the cost-offsets of avoiding locoregional and metastatic recurrences in a Spanish setting due to the addition of pertuzumab to the neoadjuvant treatment.
We developed a budget impact analysis to assess the cost-offsets of adding pertuzumab to the neoadjuvant therapy based on the reduction of metastatic and locoregional events. Cost savings have been estimated based on PFS rate at year 5. A conservative approach was considered: for locoregional recurrence, 12 months of trastuzumab adjuvant treatment was included; for mBC, direct costs (drugs, diagnosis, hospitalizations, follow-up visits) were estimated by a clinical expert panel, for first- and second-line only. Indirect costs have also been for the population under 65 years only for mBC. Clinical data used were that the addition of 4 cycles of Pertuzumab in neoadjuvant shows significant improvements in pCR and long term benefit (PFS: 5% increase at year 5).
The average cost calculated for a locoregional event was 24 and 153k€ for a mBC event (137k€ direct; 16k€ indirect). For a cohort of 100 patients, the accumulated cost-offsets for avoided events was estimated to be 636k€.
Based on PFS the benefit of adding Pertuzumab to the neoadjuvant therapy could be translated into cost-savings in further lines, that may off-set around 50% of the drug cost. This percentage should be higher when taking into consideration beyond 2 l of treatment for mBC event.

C072
BRCA2-DEFICIENT CELLS ARE HYPERSENSITIVE TO CYTOTOXICITY AND DNA DAMAGE INDUCED BY THE SOY ISOFLAVONE GENISTEIN

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Genistein is a soy isoflavone whose consumption has been associated with a decreased risk of breast cancer. However, several studies suggest that this polyphenol may increase the risk of this cancer. Cancer is a disease caused by the accumulation of DNA damage and experimental data have shown that genistein induces DNA damage in cells. This genotoxicity has been associated to topoisomerase poisoning activities and induction of double-strand DNA breaks. Since BRCA2 is an important protein implicated in repair double-strand DNA breaks and the presence of mutations in the BRCA2 gene is known to increase the risk of several cancers it is important to evaluate the cytotoxicity and DNA damage induce by genistein in BRCA2-deficient cells.
BRCA2-deficient cells (VC8) and BRCA2-complemented VC8 (VC8B2) cells were used. Cytotoxicity activity of genistein was evaluated by clonogenic survival assay. DNA damage was studied by an immunofluorescence assay in which γH2AX and its colocalization with 53BP1 foci was measured.
We found that BRCA2 deficient cells were more sensitive to the cytotoxicity of genistein. Using the focus assay and antibodies against gamma-H2AX and 53BP1 proteins, we also observed that genistein induced higher levels of gamma-H2AX. Higher levels of gamma-H2AX colocalizated with 53BP1, indicative of double-strand breaks, were also seen. Genistein induced higher levels of double-strand breaks in BRCA2-deficient cells.
These data suggest that people with genetic defects in BRCA2 may be more sensitive to the cytotoxicity and DNA damage induced by genistein. Genistein could induce carcinogenic activity, especially in people born with mutated BRCA2.

RECEPTORS

C041
AHR MODULATES ADULT NEUROGENESIS AND HIPPOCAMPAL-DEPENDENT FUNCTION

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Through dentate gyrus (DG) adult neurogenesis, new neurons integrate into hippocampal circuits driving memory processes. Adult proliferation of neural stem cells (NSCs), and integration of newborn neurons are regulated by basic helix–loop–helix (bHLH) transcription factors. AhR is a member of this family, known for mediating toxic xenobiotics effects. Several studies suggest the role of AhR role in stem cell maintenance and dendritic arborization, suggesting that AhR could be modulating hippocampal function.

P60 $AhR^{+/+}$ and $AhR^{-/-}$ C57BL6 mice were used. Contextual fear conditioning, novel object location, Barnes and Y-maze tests were used to study hippocampal-dependent deficits. A cDNA microarray was performed using hippocampus from these mice. $AhR^{+/+}$ and $AhR^{-/-}$ NSCs (Nestin/BrdU) were analyzed by flow cytometry. Hippocampal neurogenesis was studied with Ki67, DCX, NeuN, GFAP and BrdU antibodies. Images were acquired using confocal microscopy.

When compared to $AhR^{+/+}$ mice, $AhR^{-/-}$ mice showed memory hippocampal-dependent deficits in behavioural tests and impaired NSCs maintenance, dendritic arborization and axon guidance pathways. In fact, hippocampal AhR-expression was limited to NSCs, DCX and NeuN cells. The number of NSCs (Nestin/BrdU), proliferating cells (Ki67), neuroblasts (DCX) and newborn neurons ($NeuN/BrdU^{+}$) were increased in $AhR^{-/-}$. Interestingly, DCX $AhR^{-/-}$ cells showed aberrant neuroblast dendritic arborization disrupting DG circuits.

AhR is a novel modulator of adult neurogenesis and of subsequent hippocampal-dependent function.

C103
STUDY OF HOMODIMERIC 5-HT2A RECEPTORS THROUGH THE USE OF NEW HETEROBIVALENT LIGANDS

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Although the number of oligomeric G protein coupled receptors (GPCRs) described is growing every year, its implication in cell function is often unknown. Thus, the oligomers emerge as novel drug targets. Serotonin 5-HT2A receptor, a GPCR involved in diseases such as schizophrenia, has recently been reported as homooligomeric. We have hypothesized that the use of heterobivalent compounds may allow us to obtain a deeper understanding of intracelular signal transmission by 5-HT2A homodimeric receptor.

We have used two heterobivalent ligands (SY1R499 y SY1JA1469) containing a partial agonist of 5-HT2A receptor ((±) DOI) and an alkylating antagonist (phenoxybenzamine, PBZ), linked by a 11 atoms spacer and coupled to a fluorophore molecule. Both compounds bound to human 5-HT2A receptors with Ki values of 1541 and 1302 nM for SY1R499 and SY1JA1469, respectively, and showed to be agonists of 5-HT2A receptors increasing inositol phosphate formation with EC_{50} values of 2399 and 2440 nM, respectively. After washing with assay buffer, they were removed in a 65% from binding to the receptors, remaining only those compound bound by the PBZ part, but the signaling was fully abolished, suggesting that the binding of (±)DOI part bound to the free protomer of the dimer was not able to activate the receptors.

In view of the results of functional assays as well as by measuring the polarization of the fluorophore into contact with cells, we conclude that both protomers are required to induce the signaling of the oligomeric 5-HT2A receptors.

C115
CHARACTERIZATION OF NOVEL COMPETITIVE LIGANDS AT DOPAMINE D2 RECEPTOR IS IDENTIFIED BY VIRTUAL SCREENING

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The dopaminergic hypothesis of schizophrenia is a major concept explaining the ethiopathology of the disease and the effectiveness of current antipsychotics. According to this hypothesis the pathomechanism of schizophrenia is attributed to the dysfunction of dopaminergic mesolimbic (positive symptoms) and mesocortical (negative symptoms) systems. All the available antipsychotic medications are antagonists of dopamine D2 receptors and show affinity for other G protein-coupled receptors (GPCRs), in particular serotonin 5-HT2A receptors in the case of atypical antipsychotics. Hence, dopamine D2 receptor remains currently the best validated target for schizophrenia treatment. In order to identify dopamine D2 receptor antagonists, we have evaluated 21 compounds identified by structure-based virtual screening. From 21 compounds tested we found 10 D2 ligands (47.6% success rate, among them D2 receptor antagonists as designed) possessing additional affinity to other receptors tested, in particular to 5-HT2A receptors. The affinity (Ki) of the compounds ranged from 58 nM to about 24 μM. Similarity and fragmental analysis indicated a significant structural novelty of the identified compounds. Importantly, we found one D2 receptor antagonist without a protonable nitrogen atom, a key element of the classical D2 pharmacophore model and confirmed its binding mode by testing its derivatives, which are not able to interact with the conserved Asp (3.32).

C135
TLR4 MODULATES NEUTROPHIL INFILTRATION AFTER FOCAL CEREBRAL ISCHEMIA

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TLR4 plays a role on inflammation after stroke [1] but it is unknown whether participates in leukocyte infiltration and polarization. Our group demonstrated that, after stroke, the population of infiltrated-neutrophils in the brain is heterogeneous, including a population of neutrophils that express alternative phenotype N2 markers [2]. We asked whether TLR4 participates in neutrophil mobilization and polarization after stroke.

Focal cerebral ischemia was induced by permanent occlusion of the middle cerebral artery (pMCAO) in mice (2–3 months). Different groups of distal and proximal occlusion were performed by using TLR4-deficient mice (C57BL/10ScNJ) and WT (C57BL/10J). Cerebral infarct size was measured by Nissl staining and by magnetic resonance imaging (MRI) after pMCAO. Leukocyte infiltration was quantified 24 and 48 h after ischemia onset by double immunofluorescence staining and flow cytometry.

TLR4-deficient mice presented lesser infarct volumes compared to WT mice. Interestingly, we found that TLR4-deficient mice, concommitant to neuroprotection, presented an increase in neutrophil infiltration 48 h after stroke compared to WT mice by flow cytometry. Furthermore, stereological analyses revealed increased infiltrated N2 neutrophils ($Ym1^+$, $NIMP\text{-}R14^+$ cells) in the ischemic core 48 h after pMCAO in TLR4-deficient mice ($P < 0.01$; $n = 5–6$).

TLR4 modulates neutrophil polarization and its signalling may be useful for resolution of inflammation and neuroprotection after stroke.

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C143
FUNCTIONAL ACTIVITY OF THE SEROTONIN 5HT2A/MGLU2 RECEPTORS HETEROCOMPLEX IN MOUSE BRAIN

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It has been proved the existence of a 5-HT2A/mGlu2 heterocomplex in mammalian brain, identified as a possible target of hallucinogenic drugs [1, 2]. However, information about the functionality of 5-HT2A/mGlu2 complex in animal models is further unknown. Assays were performed on adult mGlu2 receptor knock-out mice ($mGlu2^{-/-}$), and wild type (WT) animals. The 5-HT2A/2C agonist (±)-DOI produced PPI-disruptive effects only in WT mice, while no effects were observed in $mGlu2^{-/-}$ mice. NMDA antagonist MK-801 disrupted sensorimotor gating processes both in WT and $mGlu2^{-/-}$ groups. Moreover, clozapine did not revert MK-801-disrupted PPI in $mGlu2^{-/-}$ mice opposing the effect observed in WT animals. Previously, it was found that the head twitch response (HTR) was not produced by the hallucinogen (±)-DOI in $mGlu2^{-/-}$ mice [3]. This response was significantly rescued in $mGlu2^{-/-}$ mice that received intra-frontal cortex injections of HSV-vectors expressing mGlu2. Some reports have implicated pertussis toxin-sensitive Gi/o proteins in the cellular responses mediated by 5-HT2A receptor activation [4]. The intracerebroventricular injection of pertussis toxin inhibited HTR in WT mice when compared to vehicle administration. Microdialysis experiments in mouse cortex showed that local (±)-DOI (300 μM)-induced increases in extracellular dopamine concentration were reduced in $mGlu2^{-/-}$ animals. This effect was partly antagonized by the co-perfusion of selective 5-HT2A antagonist M100907 in WT mice, while induced a similar increase of DA concentration than observed in $mGlu2^{-/-}$ mice. All data support the hypothesis that mGlu2 receptor is required for some behavioral and neurochemical responses induced by hallucinogenic 5-HT2A agonists.

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NATURAL PRODUCTS

C044
PROTECTIVE EFFECT OF A *QUERCUS ILEX* L. MATURE LEAVES EXTRACT IN A MURINE MODEL OF CROHN'S DISEASE

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Quercus ilex L. is one of the most commonly used plants in folk medicine to treat gastrointestinal disorders and skin infections among others.
Aims: To study the effects of an extract from mature leaves (ML) of *Quercus ilex* L. rich in phenolic compounds in acute experimental colitis model.
Colonic injury was induced by intracolonic instillation of TNBS in rats. ML was administered by gavage 48, 24 and 1 h prior to the colitis induction and 24 h later. The animals were sacrificed, 48 h after induction of colitis. Inflammation response was assessed by histology and myeloperoxidase (MPO) activity. Pro-inflammatory cytokines were evaluated by ELISA. Moreover, the protein expression of inducible nitric oxide synthase (iNOs) and cyclooxigenase (COX-2), as well as, nuclear factor e2-related factor 2(Nrf2)/heme oxigenase-1 (HO-1), mitogen-activated protein kinases (MAPKs) and nuclear transcription factor-kappa B (NF-κB) signaling pathways were studied by western blotting in colon mucosa.
Oral administration of ML extract (250–500 mg/kg) significantly reduced the degree of colonic injury and increased the amount of mucus stained by Alcian blue staining in colon mucosa. In addition, ML extract, decreased the index of neutrophil infiltration, IL-1β levels and both COX-2 and iNOS overexpression. This extract was also able to prevent the inhibitory protein IκB-degradation and inducing p65 nuclear translocation inhibition and it up-regulated the levels of the antioxidant protein Nrf2 and HO-1.
Quercus ilex L. extract reduces the damage in a rat model of Crohn's disease, and returns pro-inflammatory markers to basal levels probably through NfκB and Nrf2/HO-1 signally pathways.

C052
DETERMINATION OF THE CELL VIABILITY IN HUMAN PROSTATE CANCER CELLS BY SIDERITIS HYSSOPIFOLIA

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We demonstrated that Sideritis hyssopifolia has antioxidant properties and therefore, this plant shows potential therapeutic benefits. The aim of the study was to evaluate the effects of the ether, methanol and chloroform extracts obtained from the aerial parts of Sideritis hyssopifolia, on cell viability in human prostate cancer cells, using MTT assay.
LNCaP cell line were seeded (104 cells/well) in 100 µl of culture medium. After 24 h, the cells were treated with 200 µl of each extract dissolved in culture medium (0.1% DMSO) and incubated at 37°C for 24, 48 and 72 h. Control- treated (0.1% DMSO) cell and control-untreated cell were incubated with culture medium. At the end of treatments the medium was removed, cells were washed with PBS, and 100 µl of MTT solution (5 mg/ml) was added. After 3 h incubation at 37°C, the formazan crystals were solubilized in 50 µl of SDS (20%) and the plates were incubated overnight at room temperature. Then, the absorbance was measured at 560 nm.
The results were expressed as the percentage of viable cells in comparison with the control cells, for which the cell viability was 100%. The percentage of cell viability tested at 24, 48 and 72 h were for ether extract: 65.122, 44.918 and 14.313; for methanol extract: 55.11, 53.186 and 19.493 and for chloroform extract: 61.254, 51.359 and 6.666, respectively.
LNCaP cells exhibited a time-dependent inhibition of the cell viability. The highest inhibition of the cell viability was observed for chloroform extract at 72 h.

C054
HYPOCHOLESTEROLEMIC EFFECTS OF SIDERITIS HYSSOPIFOLIA IN RABBITS

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Since several years ago, hypercholesterolemia is considered to be one of the major contributors in progression of atherosclerosis. Sideritis hyssopifolia is a vegetal species endemic subspecies from some mountain changes with demonstrated antioxidant properties. In Spain this plant is endemic from Cantabric chain and Pirinees, where it has been traditionally used as an aromatic and digestive plant. The purpose of this study is to determine the hypocholesterolemic action of Sideritis hyssopifolia in rabbits.
4 groups of 10 animals each were used. The animals of group 1 were used as control, and received a standard chow. The rabbits of the other groups were fed with special diets: number 2 received a standard feedingstuff containing a 0.20% of cholesterol, for number 3 the chow was enriched with cholesterol 0.2% and Sideritis hyssopifolia (2.36 g/100 g of feedingstuff) and group number 4 received a diet containing 0.20% of cholesterol and simvastatin at a 20 mg/kg dosage. After 10 weeks of treatment, total-cholesterol, HDL and LDL were determined.
The mean values for total cholesterol in the four groups studied were: 58.7, 565.1, 442.1 and 418.6 mg/dl, respectively. HDL and LDL concentrations were also lower in the groups that receives Sideritis hyssopifolia and simvastatin than in the group that was fed with the diet enriched in cholesterol (68.5, 71.8 and 85.7 mg/dl for HDL and 168.3, 152.8 and 214.0 mg/dl for LDL)
Sideritis hyssopifolia reduced total cholesterol, HDL and LDL levels in a similar way as simvastatin

C056
EFFECTS OF SIDERITIS HYSSOPIFOLIA ON TRIGLYCERIDES, APOLIPOPROTEIN B AND ATHEROGENIC INDEX IN RABBITS

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Sideritis hyssopifolia, is a little woody plant belonging to Labiatae family typical from humid and mountain habitats. Several flavone-gly-

cosides have been isolated from this plant and these compounds have been reported to have antioxidant properties. The aim of this study is to determine the effects of this plant on triglycerides, apolipoprotein B and atherogenic index in rabbits

To carry out the study four groups of rabbits were used. Group 1 was used as control, and received a standard feedingstuff. Groups 2, 3 and 4 were fed with the same feedingstuff but enriched with a 0.20% of cholesterol. The diet of group 3 also contained Sideritis hyssopifolia (2.36%) and that for group 4 simvastatin (20 mg/kg). After 10 weeks of treatment, triglycerides, apolipoprotein B and atherogenic index were determined.

The mean values for triglycerides were very similar in the four groups studied: 67.4, 68.5, 77.6 and 79.9 mg/dl, respectively. Apolipoprotein B concentrations were lower in the groups receiving Sideritis hyssopifolia and simvastatin (10.5 and 24.0 mg/dl, respectively) than in the group fed with the diet enriched in cholesterol (41.4 mg/dl) and similar to those determined in the control group (17.9 mg/dl). Similar results were obtained for the atherogenic index, which values were 2.46, 5.98, 3.16 and 2.76, respectively in the four groups.

Neither Sideritis hyssopifolia nor simvastatin modified triglyceride levels in rabbits fed with a diet enriched with cholesterol. However, they reduced apolipoprotein B and atherogenic index levels in a similar way.

C097
DIOSMETIN AND DIOSMIN: CHANGES IN PLATELET STRUCTURE AND ACTIVITY

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Cardiovascular diseases are major causes of death in the developed world. Platelets play a key role in hemostasis and in the initiation and propagation of thrombus formation. Anti-platelet therapies are used widely, but current are associated with side effects including problem bleeding. Drugs from medical plants with vascular protection properties such as flavonoids could exert an important role in the platelet function regulation.

Blood was extracted from 10 free-drug normal volunteers. Drugs (flavonoids: diosmetin and diosmin) were dissolved in dimethyl sulfoxide and added to the samples blood with or without calcium ionophore. Flow cytometry and fluorescence microscopy were employed to detect, using microbeads, the characterization of platelet-derived microparticles, platelets, and aggregates generated by *in vitro* activation of platelets using calcium ionophore.

Both flavonoids showed certain antiplatelet activity, compared to the control, reducing the potent effect of the pro-aggregant agent. A percentage antiplatelet activity index (ACI) of $29.48 \pm 3.21\%$ was recorded for diosmin 2 mM, and $13.51 \pm 2.36\%$ for diosmetin 2 mM achieved descended the 10% of the PDMPs generated by the calcium ionophore, therefore this product proved a moderate antiplatelet capacity index. These drugs could have a potentially use in the prevention of cardiovascular events, taking to account the differences in the chemical structures of the heteroside and the aglycon here studied might explain their different ACIs.

C120
NEW TETRAHYDROISOQUINOLINES BEARING CARBAMATES AS DOPAMINERGIC LIGANDS

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Dopamine neurotransmission plays an important role in neurological and psychiatric disorders like Parkinson's disease and schizophrenia. Therefore, this research is being focused on the development of new dopaminergic ligands.

New N-methyl-1-pentyl-6,7-dihidroxy-1,2,3,4-tetrahydroisoquinolines bearing halogenated phenylethylamine- or phenylamine-carbamate moieties were synthesized. The binding affinity for dopaminergic receptors D1 and D2 on striatal membranes was evaluated. In addition cytotoxicity studies of the most active compounds were carried out in human umbilical venous endothelial cells (HUVEC).

Seven new catechol tetrahydroisoquinolines bearing a carbamate group with phenylethylamine- or phenylamine-substituents were successfully synthesized. All catechol and methoxylated compounds were able to display affinity for D1 and D2 dopaminergic receptors at micromolar or nanomolar range. The catechol tetrahydroisoquinoline 7a displayed the highest affinity (Ki = 91 nM) and compound 4 the greatest selectivity (Ki D1/D2 ratio = 16.3) for D2. Compound 7a and 8a showed significant cytotoxicity but only at 100 μM on the tested human cells. The presence of a catechol group at the isoquinoline core improved the affinity for dopaminergic receptors. The introduction of a phenylethylamine or a phenylamine through a carbamate group increased the affinity towards D2 receptors. In general, halogenation in para position of the romaticring substituents worsened the affinity towards D2, but improved that towards D1 receptor. A. Galán was the recipient of a predoctoral fellowship from FPU program of Spanish Ministry of Education. This work was financially supported by the Spanish Ministry of Economy and Competiveness (SAF2011-23777).

C141
RESEARCH STUDY: EFFECTIVENESS OF THE USE OF ALOE VERA AS A PROCEDURE FOR PRESSURE ULCERS

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The Aloe Vera is a natural method that has been used since ancient times to induce scarring, treating burns, psoriasis, wounds of various etiologies well as other conditions and may be an alternative therapy for pressure ulcers.

The aim of this study is to present an update on the effects of Aloe Vera as a therapy for the treatment of these ulcers information.

The search was performed using primary and secondary sources of information.

And it has been limited to articles in Castilian, English and Portuguese, published between 2004 and 2014. We used the descriptors in Health Sciences Aloe Vera (Aloe); Pressure ulcer (Pressure Ulcer).

After the literature search a total of 85 articles were obtained.

The found literature showed various benefits of using Aloe Vera as its low price, easy application and accessibility to all users.

Organizations such as the World Health Organization emphasized that medicinal plants can be a good source for obtaining a variety of products to treat various skin diseases.

Regarding treatment of pressure ulcers grade I and II, the evidence found confirmed that Aloe Vera is effective for healing, but no references to justify their use in ulcers of grade III and IV were found.

It has shown an improvement of pressure ulcers grade I and II in patients treated with therapy Aloe Vera, therefore it would be justified for the treatment of these skin lesions.

PARMACOKINETICS

C087
ULTRAFLEXIBLE LIPOSOMES AS PERMEABILITY ENHANCERS FOR CYCLOSPORIN-A SKIN ABSORPTION

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Inflammatory skin diseases such as psoriasis and atopic dermatitis, affect a high, percentage of the population and the current available therapies can only reduce the symptoms, but not the causes. Cyclosporine A, is a very effective drug for this skin illnesses. However, it is used as a second line therapy because of its serious systemic adverse effects. Topical administration is limited in this case by the inability of ciclosporine to diffuse and distribute through and within the epidermis to reach the dermis.
To facilitate the permeability of this drug, ultraflexible Cyclosporine-A liposomes have been formulated and the ability to diffuse through the stratum corneum and dermis access have been evaluated.

Two types of ultraflexible liposomes containing, Tween 20 and Tween 80, have been prepared by film-method followed by extrusion or sonication to reduce their size and homogenize particle dispersion. Ethosomes were prepared by the Touitoui method followed by extrusion. The permeability tests were performed using heat separated epidermis obtained from human abdominal skin, by means of Franz diffusion cells.
Liposomes increase the solubility of cyclosporine in aqueous medium and facilitate the diffusion of drug through skin. After 1.5 h, detectable concentrations of Cyclosporine were found in the receptor compartment. The profiles show access to deeper skin layers but no steady state conditions are reached, meaning that it is a controlled process, except for the ethosomes.
These results allow to predict a liposome-mediated increase of drug concentration at the target site, while reducing the risk of systemic side effects.

AUTHOR INDEX