04. Inflammation and Immunopharmacology

04.001 Skin wound healing properties of gold nanoparticles: A preliminary study. Ventura ACSSB, Ferreira GK, Soley BS, Ferreira JCP, Otuki MF, Cabrini DA UFPR – Farmacologia

Introduction: Wound healing occurs in order to restore skin barrier. This process involves a coordinated sequence of interactions and reactions between molecules and cells and it is didactically divided into three phases: inflammation, proliferation and maturation. Gold nanoparticles (AuNPs) have been researched in biomedical field because they are able to reduce angiogenesis, inflammatory cells infiltration, cytokines and growth factors release. Since anti-inflammatory properties and stimulation of keratinocytes proliferation may be useful during the wound healing process, the aim of this study is to evaluate the influence of AuNPs on inflammatory skin model, on keratinocytes viability and proliferation and on wound closure in mice. **Methods**: 10 nm AuNPs were synthesized by Turkevich *et al.* method (1951) and embedded in non-ionic cream at 0.1% for topical application or in saline at 2.5 mg/kg for i.p. injection or v.o. gavage. Mice ear edema was induced with TPA followed by treatment with AuNPs (i.p., v.o. or topical) or dexamethasone (as positive control, topical or v.o.). A digital micrometer was used to measure ear thickness 6 and 24 h after TPA. Animals were euthanatized and MPO activity was determined. In the excision experiment, a skin excision using a 6 mm punch was performed with animals under deep anesthesia induced by ketamine and xylazine. Once a day each wound was treated, measured and photographed. *In vitro*, viability and proliferation of keratinocytes submitted to AuNPs (2.5, 10, 30 and 70 mg/L) were analyzed using MTT assay. Data analysis was done using Image J software and Prism. **Results**: Treatment with AuNPs i.p. and topical caused an inhibition of ear edema at both time point evaluations. At 6 h, AuNPs i.p. and topical inhibited 41.87+-0.012% and 43.14+-0.006% of ear edema, respectively. At 24 h, the inhibition was 90.34+-0.003% and 76.88+-0.005%, respectively. Oral treatment with AuNPs did not interfere significantly in edema or in MPO activity. Topical treatment, as well as i.p. injection, however, caused a decrease in the MPO activity of 40.15+-0.006% and 25.27+-0.004%, respectively. In wound healing, no significant difference was noted between groups treated with AuNPs i.p. or topical and control (vehicle) until the 11^{th} day after excision. From the 11^{th} day on, the group treated with AuNPs i.p. presented a faster closure, when compared to other groups. Considering MTT reaction, keratinocytes treated with BFS 10% and post-treated with AuNPs at all concentrations exhibited less viability and proliferation than control. At 70 mg/L, AuNPs reduced 88.84+-2.49% of keratinocytes proliferation. Conclusion: Topical and i.p. administration of AuNPs may possess anti-inflammatory properties, and could facilitate wound healing (i.p.), but probably acting on mechanisms that not involve keratinocytes proliferation. However, further investigations are needed in order to clear its effect in other skin cells and determine the security and efficacy of AuNPs formulations for wound healing. References: Turkevich, J. *et al*. Disc Far Soc. 11, 55, 1951; Pivodová, V. *et al*. Nanobiomedicine 2, 1, 2015; Tsai, C. *et al*. Arthritis & Rheumatism 56(2), 544, 2007. This work was supported by CAPES. The experimental design was approved by CEUA-Bio of UFPR, number 610.

04.002 Inosine antiproliferative effect on keratinocytes in culture. Silva CD[1], Soley BS[1], Pawloski PL[1], Santos ARS[2], Cabrini DA[1] [1]UFPR- Farmacologia, [2]UFSC – Fisiologia

Introduction: Inosine is an endogenous purine that acts as extracellular signaling molecular and its effects are associated with the action on adenosine receptors. It has been shown that inosine has an important anti-inflammatory activity, being capable of reducing the levels of pro-inflammatory cytokines (TNF-α, IFN-γ and IL-1) and increase the expression of anti-inflammatory cytokines (such as IL-10), as well as to reduce macrophages and neutrophils influx. Our group has previous observed that topical application of inosine interfered in different events in animal models of skin inflammation (de Oliveira et al., Purinergic Signalling, 2016, submitted). Therefore, this study aimed to investigate the effect of inosine on keratinocytes. **Methods**: Human keratinocytes HaCaT cell line were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing fetal bovine serum 10% and 1% penicillin/streptomycin at 37 °C with 5% CO_2 in a humidified atmosphere. After treatment with different concentrations of inosine (0.1 to 30 mM) for 24 h, cell viability was assessed by MTT (3- (4, 5-dimethylthiazol-2-yl) -2,5diphenyltetrazolium bromide) and neutral red assay. To evaluate the activity of inosine on cell proliferation, MTT tests were carried out in 72 h and quantification of proliferating cell nuclear antigen expression (PCNA) by immunohistochemistry were performed. **Results**: In both viability methods, none of the tested inosine concentrations were able to cause significant reduction in keratinocytes viability. However, in cell proliferation protocol, the concentrations of 3, 10 and 30 µM of inosine were able to significantly reduce this parameter by 33.2 ± 4.1%; 67.6 ± 5.3% and 81.3 ± 3.6%, respectively. Similarly, the presence of inosine 10 µM reduced PCNA expression in 44.7 ± 7.5% when compared to the control group. Conclusion: These results indicate that exogenous inosine acts directly on keratinocytes and it is promising for reducing the keratinocytes proliferation, being interesting option for treatment of hyperproliferative skin diseases, such as psoriasis. Thus, further investigation concerning the mechanism of action is in progress. Acknowledgment: CNPq and CAPES.

04.003 Impaired cytokine release by bone marrow derived macrophages from diabetic mice is related to high glucose environment. Ayala TS, Tessaro FHG, Bella LM, Martins JO
FCF-USP – Análises Clínicas e Toxicológicas

Introduction: Diabetic patients are more susceptible to infections, due mainly to a deficiency in immune response. Hyperglycemia is one of the triggers to this event and a high glucose environment may lead to modifications in macrophages response. Here we hypothesized if a high glucose environment alters cytokine release by bone marrow derived macrophages (BMM). **Methods:** To this purpose, we use BMM from diabetic (alloxan 60 mg/kg, i.v.) and no diabetic (saline) male C57BL/6 mice (***CEUA/FCF/USP – 488).*** The cells were maintained in 3 different glucose concentrations such as normal glucose (NG) (5,5 mM) and high glucose (HG) (25 and 40 mM) and stimulated or not, by LPS (100 ng/mL) for 3, 6, 12, 24 and 48 hours. Cells viability was measured by Trypan blue and toxicity of the treatments were evaluated by MTT assay (24 hours). Cytokine released were measured by enzyme linked immune assay. **Results**: After 6 and 12 hours, BMM from no diabetic mice produced more TNF-α and IL-10 compared to diabetic BMM. Although, after 3, 12 and 24 hours, diabetic BMM produced more IL-6 compared to BMM from healthy mice. In diabetic BMM a HG environment lead to minor amounts of TNF-α, but higher levels of IL-6 compared to normal glucose medium. Still, HG increased IL-10 released both from healthy and diabetic BMM. **Conclusion:** Our findings demonstrate that hyperglycemia and HG environment affects macrophages on cytokine release. **Financial support:** FAPESP (2014/05214-1), CAPES and CNPq.

04.004 Insulin enhances LPS-induced cytokines and signaling pathways in bone marrow-derived macrophages from diabetic mice. Tessaro FHG, Ayala TS, Bella LM, Nolasco EL, Martins JO FCF-USP – Análises Clínicas e Toxicológicas

Introduction: Diabetic patients are more susceptible to infections, this event occur due to impaired immune response. Insulin exerts a powerful effect in immune cells from diabetic mice. We have hypothesized if insulin may act on cytokine release in diabetic macrophages stimulated by lipopolysaccharide (LPS). We investigated the effect of insulin on the LPS-induced production of tumor necrosis factor (TNF)-α, interleukin (IL)-6, on the expression of p38, JNK, ERK 1/2 and Ak in diabetic bone marrow-derived macrophages (BMM). **Methods:** To this purpose, BMM from male diabetic C57BL/6 mice (alloxan 60 mg/kg, i.v., ***CEUA/FCF/USP - 467***) were stimulated by LPS (100 ng/mL) for 3, 6 and 12 hours. Cell viability was determined by Trypan blue and toxicity of the treatments was evaluated by MTT assay (24 hours). Insulin (1 mU/mL) was added alone or simultaneously with LPS. Expression of phosphorylated p38, JNK, ERK 1/2 and Akt were assessed by immunoblotting in cell lysate after 1 hour of treatments. Pharmacological inhibitors of PI3-kinase, LY-294002 (10μM) and wortmannin (10 nM) were added concomitant with the insulin and LPS. **Results**: Relative to Control, LPS induced a significant increase release of cytokines (TNF-α and IL-6) and in the expression of p38, JNK, ERK 1/2, and Akt. Compared to LPS, LPS-Insulin treatment increased these cytokines and in the expression of p38, JNK, ERK 1/2 and Akt. Insulin treatment alone did not activated the expression of p38, JNK, ERK 1/2 and Akt or released cytokines. PI3-kinase inhibition by wortmannin decreased TNF-α release, and the inhibition by LY294002 decreased both TNF-α and IL-6 after LPS-Insulin treatment. **Conclusion:** These results show that in BMM stimulated by LPS, insulin enhanced TNF-α and IL-6 secretion through up-regulation of p38, JNK, ERK and Akt, which suggest a key role of insulin in the macrophage immune response through PI3-kinase pathways. **Financial support:** FAPESP (2014/05214-1), CAPES and CNPq.

04.005 Vitamin D modulates lipopolysaccharide-induced immune response in raw 267.4 macrophages. Bella LM[1], Quirino TC[1], Tessaro FHG[1], Nolasco EL[1], Ayala TS[1], Azevedo CB[2], Martins JO[1] [1]FCF-USP – Análises Clínicas e Toxicológicas, [2]Unifesp

Introduction: Macrophages are key cells in infectious and inflammatory processes. The magnitude of these phenomena can be regulated by modulation of cytokine release. Immunomodulatory effect of vitamin D has been assigned by the activated form, as known as calcitriol. However, the cholecalciferol, an inactive form of vitamin D, activity is not described as an immunomodulatory agent. Thus, this study evaluated the effects of cholecalciferol in the release of interleukin (IL) 1β, IL– 6, IL-10 and nitric oxide (NO) production in RAW 264.7 macrophages. **Methods:** Murine RAW 264.7 macrophages were cultured in hormone-free medium (DMEM) supplemented with penicillin (40 U/mL) and streptomycin (50 µg/mL). Cells ($2x10^6$) were plated in 12-well plates for 4h and 24 h at 37°C with 5% CO_2 atmosphere and divided in four groups (n= 6, duplicate): control; LPS (100ng/mL); vitamin D (100nM/mL); LPS + vitamin D (100nM/mL). IL 1β, IL-6 and IL-10 were measured by ELISA; NO by Griess and cell viability by MTT assay. **Results:** Cell viability not showed differences between the groups. When compared IL-1β, IL-6, IL-10 and NO released, there were no differences between cells threated with cholecalciferol and control group after 4h and 24h. However, cells stimulated with LPS released more IL 1β, IL-6, IL-10 and NO than control group after 4h and 24h of stimulation. Interestingly, cells stimulated with LPS and threated with cholecalciferol released less IL 1β, IL-6 and NO than cells stimulated with LPS after 4h (IL1β: 227.4+15.37 vs 170.4+37.11, $p<0,01$; IL-6: 388.8+32.47 vs 313.9+14.39, $p<0,001$) and 24h (IL1β: 129.2+24.39 vs 65.52+15.79, $p<0,001$; IL-6: 985.8+38.55 vs 814.5+53.58, $p<0,001$ and NO: 47.55+6.58 vs 28.67+3.69). When compared IL-10 released, there were no differences between cells stimulated with LPS and threated with cholecalciferol and cells stimulated with LPS after 4h and 24h. **Conclusion:** These results suggest that cholecalciferol might modulate the release of IL 1β, IL-6 and NO by RAW 267.4 cell. **Financial support:** FAPESP (2010/02272-0 and 2014/05214-1); CNPq (470523/2013-1) and CAPES.

04.006 Protective effect of gedunin on TLR-mediated inflammation by modulation of inflammasome activation and cytokine production: evidence of a multitarget compound.

Borges PV[1], Moret KH[1], Manjunathaiah RN[2], Costa TEM[1], Monteiro AP[3], Carneiro AB[3], Pacheco P[1], Temerozo JR[4], Habib DCB[4], Henriques MG[1,5], Penido C[1,5] [1]Farmanguinhos-Fiocruz – Farmacologia Aplicada, [2]Osmania University – Pharmaceutical Chemistry, [3]IOC-Fiocruz – Imunofarmacologia, [4]IOC-Fiocruz – Imunologia, [5]CDTS-Fiocruz

Introduction: Recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) by macrophages (Mø) results in the activation of different signaling cascades, resulting in cytokine production and inflammasome activation. Previous data from our group have demonstrated that the natural limonoid, gedunin, a known Hsp90 inhibitor, presents marked anti-inflammatory effects in vitro and in vivo (Borges et al., Mol Pharmacol 88:949, 2015; Conte et al., Molecules 20(2):2636, 2015; Ferraris et al., Int Immunopharmacol. 14:82, 2012). Here, we demonstrate the modulatory effect of gedunin on TLR activation in vitro and in vivo. **Methods and Results**: Intraperitoneal (i.p.) pre- and post-treatments of C57BL/6 mice with gedunin (0.5 mg/kg) impaired the pleural influx of mononuclear cells, eosinophils and neutrophils, as well as the production of tumor necrosis factor (TNF)-α, interleukin (IL)-6 and nitric oxide (NO), triggered by the intra-pleural (i.pl.) injection of lipopolysaccharide (LPS, 250 ng/cavity). Accordingly, in vitro post-treatment of Mø with gedunin (10 µM) also impaired LPS (50 ng/ml)-induced production of such mediators, as assessed by ELISA. Gedunin diminished LPS-induced expression of the nucleotide-binding domain and leucine-rich repeat protein-3 (NLRP3) on pleural leukocytes in vivo and in Mø in vitro, as revealed by western blot (WB) and immunofluorescence. In line with these results, gedunin inhibited LPS (1 µg/ml) plus ATP (2 mM)-induced caspase-1 activation in Mø (evaluated by FAM-FLICA Caspase-1 Assay Kit) and impaired the production of the inflammasome-related cytokine IL-1β in vitro and in vivo. We also demonstrate that gedunin effect is not restricted to TLR4 signaling, since this compound suppressed TNF-α production by macrophages stimulated with the TLR2 and TLR3 agonists, palmitoyl-3-Cys-Ser-(Lys)4 (PAM3, 1 µg/ml) or by polyriboinosinic:polyribocytidylic acid (POLY I:C, 1 µg/ml) in vitro. In addition, gedunin treatment triggered the generation of the anti-inflammatory factors IL-10 and heme oxigenase-1 (HO-1), assessed by ELISA and WB, at resting conditions or upon TLR4, TLR3 and TLR2 stimulation. Moreover, in silico modeling studies revealed that gedunin efficiently docked into caspase-1, TLR2, TLR3 and to the component of TLR4 complex, myeloid differentiation protein-2 (MD-2). Conclusion: Gedunin is a multitarget compound with anti-inflammatory properties, capable to modulate TLR2, TLR3 and TLR4 signaling, by impairing inflammasome activation, production of inflammatory mediators and leukocyte mobilization, as well as by triggering the production of anti-inflammatory factors. **Financial support**: FIOCRUZ/CNPQ/CAPES/FAPERJ. This study was approved by Committee on Ethical Use of Laboratory Animals of Oswaldo Cruz Foundation (#LW-62/12).

04.007 Maresin-1 and its role as a hepatoprotective against diethylnitrosamine-induced liver fibrosis in Sprague-Dawley Rats. Rodriguez MJ[1], Dominguez KA[1], Donoso WK[2], Zuñiga Hernandez J[1], Beltran OA[1] [1]University of Talca – Medical Research, School of Medicine, [2]University of Talca – Oral Pathology, School of odontology

Introduction: Maresin 1(MaR1) was recently reported to have protective properties in several different animal models of acute inflammation by inhibiting inflammatory response. However, its function in acute liver injury is still unknown[1]. MaR1 is a lipid derivative of the polyunsaturated fatty acids omega-3 (EPA and DHA). In particular, DHA, which is significantly involved in improving liver damage due to its anti-inflammatory and antioxidant role. MaR1 has shown an effect on regeneration as well as a potent anti-inflammatory and pro-resolutive activity[2,3]. Given the health benefits of MaR1, the aim of this work is to study the hepatoprotective effect of MaR1 on the progression of diethylnitrosamine (DEN)-induced hepatic fibrosis in an animal model. **Methods:** Sprague-Dawley rats were induced with hepatic fibrosis by DEN administration (70 mg/2 mL/kg) intraperitoneally (i.p) once a week for a period of 4 weeks. All the groups were treated with MaR1 (70 ng/animal) or vehicle i.p (ethanol 0,025% in 0,9% NaCl) twice a week. At the end of treatment blood samples were taken from the heart and liver tissue for biochemical and histopathological analysis. Plasma levels of transaminases (AST and ALT) were determined by specific kit (Valtek, S.A., Chile). The inflammatory response mediated by TNF-alpha levels was determined by ELISA kit (Thermo, Meridian, Rb, USA). Fibers collagen deposition was determined by Masson's Trichrome stain[4]. Comparisons between groups were performed by one-way ANOVA followed by Tukey's test, and $p<0,05$ was considered as significant (GraphPad Prism versión 6.0, software GraphPad, San Diego, CA, EE.UU.). **Results:** A decrease in transaminase levels (AST and ALT) was observed in the groups treated with MaR1-DEN when compared with the fibrosis (DEN) group. Along with the above, it is possible to observe a normalization of cytokine levels of TNF-alpha in the group MaR1-DEN compared with the unprotected animals (DEN alone) and the control group (CC). The histophatological analysis showed that the collagen fibers were located near the central vein in the DEN group, where was observed an increased deposit of extracellular matrix. This situation is reverted in the animals that received MaR1 with the DEN administration. **Conclusion:** This study suggests that the administration of MaR1 will promote the resolution and stop progression of liver fibrosis in a rat model. **References**: [1]Li, R., Oxid Med Cell Longev., 2016, 2016. [2]Serhan, C., J Periodontol., 79(8S), 1520, 2008. [3]Serhan, C., The FASEB Journal, 26(4), 1755-1765 [4]Howat, WJ., Métodos , 70 (1), 12, 2014 **Financial support**: Financed by CONICYT-PCHA / National Doctoral / 2015-21151622 Certificate of approval by the ethics committee (CIECUAL), Nº Folio: 2016-06-B

04.008 Maternal Obesity Programs the OVA-induced Airway Inflammation in the male offspring. E-Lacerda RR[1], Bordin S[2], Antunes E[1], Anhê GF[1] [1]Unicamp – Farmacologia, [2]USP – Fisiologia e Biofísica

Introduction: Asthma is respiratory disease hallmarked by intermittent airway obstruction followed by an inflammatory phase (Verstraelen et al., 2008). Allergic asthma, the most common type of asthma, can be triggered by exposure to a myriad of environmental antigens such as air dust, pollens and food components. Multiple characteristics are likely to predispose to asthma development, including those acquired during the fetal development and early post-natal life (Camilo et al., 2010). It is noteworthy that recent large-scale epidemiological data shows that maternal obesity has a positive correlation with asthma incidence in the progeny. The aim of the present study is to determine if maternal obesity in mice programs the airway inflammation in the male offspring. **Methods:** Female C57/B6Junib mice fed with a high fat diet (HFD) or standard chow (SC) for 4 weeks before mating. These diets were kept throughout mating (4 days), pregnancy and lactation. After weaning, the two groups of male offspring were fed with SC ad libitum. By reaching 8 weeks of life, half of the male offspring was assigned to sensitization with ovalbumin (OVA) while the other half was exposed to vehicle. Thereafter, all mice received an acute challenge with OVA 24 hours prior sacrifice. Broncho alveolar lavage (BAL) was collected for absolute and differential counting of leucocytes and interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 10 (IL-10) and tumor necrosis factor alpha (TNF-□□ determination. Lungs were processed for eosinophil peroxidase (EPO) and myeloperoxidase (MPO) activities. Serum was collected for immunoglobulin E (IgE) determination. **Results:** HFD fed mothers had increased body and perigonadal fat pad weights and triglycerides, cholesterol and glucose levels as compared to SC fed mothers (respectively 16, 85, 28, 33 and 25%). These parameters were similar between male mice born to HFD and SC mothers. OVA-sensitized mice born to HFD mothers had reduced total leucocytes and eosinophil counts in BAL (respectively 52 and 98 % lower than OVA-sensitized mice born to SC mothers). Accordingly, lung tissue from OVA-sensitized mice born to HFD mothers had reduced EPO and MPO activity (respectively 71 and 28%). On the other hand, OVA-sensitized mice born to HFD presented a trend towards increase in IL-4, IL-5, IL-10 and TNF□ in BAL and increased circulating IgE levels as compared to OVA-sensitized mice born to SC mothers (70%). **Conclusions:** The high levels circulating IgE seen in mice born to HFD mothers suggest that maternal obesity predisposes to increased Th2 immunity in the male offspring. In spite of this adaptation, inflammatory response in the offspring born to HFD mothers had reduced eosinophil mobilization to the respiratory tissue. **Financial support:** FAPESP Animal Research Ethical Committee CEUA protocol number 3875-1. **References:** CAMILO, D. F. et al. Jornal de Pediatria, v. 86, p. 6, 2010. VERHASSELT, V. et al. Nature medicine, v. 14, p. 170, 2008.

04.009 Anti-Inflammatory, analgesic and vasorelaxant activities of new pyrazole derivative 5-[1-(4-fluorphenyl) -1H-pyrazol-4-yl]-2H-tetrazole. Oliveira LP[1], Silva DPB[1], Florentino IF[1], Fajemiroye JO[2], Oliveira TS[1], Ghedini PC[1], Menegatti R[3], Costa EA[1] [1]UFG – Farmacologia, [2]UFG – Ciências Farmacêuticas, [3]UFG – Farmácia

Introduction: The inflammatory process is a natural physiological response towards the removal of harmful agents and reparation of lesioned tissues. Pain, one of the classic signs of inflammation, is considered as an adaptive mechanism of alert that triggers appropriate protective responses to real or imminent injury. However, at a certain point both pain and inflammation lose the purpose of alert and protection of the organism, and begin to influence significantly the lifestyle of millions of people worldwide. Pyrazole compounds are known to possess antipyretic, analgesic and anti-inflammatory effects. The molecular modifications or synthesis of compounds vital to drug discovery with desirable pharmacological. This study sought to evaluate the analgesic, anti-inflammatory, vasorelaxant effects and action mechanisms of 5-[1-(4-fluorphenyl)-1*H*-pyrazol-4-yl]-2*H*-tetrazole – FPPT – a new pyrazole derivative. **Methods:** Analgesic activity: Acetic acid-induced abdominal writhing, formalin-induced pain, tail flick test; anti-inflammatory activity; carrageenan-induced paw edema, carrageenan-induced pleurisy. The effects of FPPT on nitric oxide pathway, prostaglandin and ion channels were investigated using isolated organ model. **Results:** In the acetic acid-induced abdominal writhing test, treatments with FPPT (9, 18 and 36 mg/kg p.o.) reduced the abdominal writhing. In the formalin test, FPPT 36 mg/kg reduced the licking time in both neurogenic and inflammatory without showing antinociceptive effect in the tail-flick test. In addition, the carrageenan-induced paw edema and cell migration in the carrageenan-induced pleurisy were reduced by FPPT. It was observed that FPPT has vasorelaxant effect that was attenuated by L-NAME, ODQ, TEA or GB. FPPT also blocked $CaCl_2$ induced contraction in a dose-dependent manner. **Conclusion:** The compound showed anti-inflammatory, analgesic activity and vasorelaxant effect that involve the NO/cGMP pathway and K+ channels. This results partially explain peripheral analgesic activity of FPPT. . The experimental protocols were approved by the Ethic Commission of UFG (N^{o}137/2009, 017 and 020/2013).

04.010 Exogenous and endogenous hydrogen sulphide protects against histaminergic and nonhistaminergic pruritus and inflammation in mice dorsal skin. Rodrigues L[1], Schmidt TP[1], Florenzano J[1], Cerqueira ARA[1], Teixeira SA[1], Wood ME[2], Whiteman M[2], Muscará MN[1], Costa SKP[1] [1]ICB-USP – Farmacologia, [2]University of Exeter

Introduction: Pruritus, similarly to pain, is a sensory modality acting as a body protective mechanism. Despite anti-pruritic advances made with histamine antagonists and topical glucocorticoids therapy, itch can be very often and intractable condition by these drugs (Leslie, Handb Exp Pharmacol. 226:337, 2015), thus research focusing on pruriceptive mechanisms is still required. The 'gasomediator' hydrogen sulfide (H_2S) is involved in nociceptive mechanisms, but its effect on pruriception is poorly known. The effect of slow-releasing H_2S donor (GYY4137) in histaminergic and nonhistaminergic-induced pruritus and associated inflammation in mouse dorsal skin was tested. **Methods**: Experiments were performed in male BALB/c mice (25-30g) or Wistar rats (180-200 g). Under isoflurane anesthesia, the mouse dorsal skin was shaved and either compound 48/80 (C48/80; 3 µg/site), histamine (1 µmol/site) or chloroquine (100 µg/site) diluted in Tyrode was intradermally (i.d.) injected (0.05 ml) alone or in addition to increased doses of GYY4137 (0.3-10 nmol/site) and the scratching behavior recorded, results were analyzed as scratching bouts measured in 40 min (Costa, Vascular Pharmacology. 45, 209. 2006). Skin plasma protein extravasation and neutrophil influx was assessed by the extravascular accumulation of i.v. injected ^{125}I-albumin and increased myeloperoxidase (MPO) activity in the skin, respectively. The endogenous H_2S role was assessed by the pretreatment of animals (-60 min; i.p.) with the CSE or CBS inhibitor β-cyanoalanine (BCA, 50 mg/kg) or aminooxyacetic acid (AOAA, 20 mg/kg), respectively. High-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) was used to measure histamine release from C48/80-induced rat mast cell degranulation in vitro. Data are as mean ± SEM. Stats were performed by ANOVA followed by Dunnett's test. $P<0.05$ was taken as significant. **Results**: C48/80, histamine or chloroquine significantly increased itching frequency compared to tyrode. Histamine or chloroquine-induced pruritus was significantly inhibited by 72 ± 7 % and 66 ± 5 %, respectively with GYY4137 co-injection (1 nmol/site; n=5-8), whereas the co-injection of GYY4137 with C48/80 did not. GYY4137 (1–100 nmol/site; n=6-10) ameliorated C48/80-induced plasma extravasation and decreased the MPO activity, but the inhibition of endogenous H_2S synthesis exacerbated C48/80-induced pruritus and MPO activity. HPLC-MS/MS assay revealed that fast-releasing H_2S donor (Na_2S and Lawesson's reagent), but not GYY4137, significantly attenuated C48/80-induced histamine release from mast cell in vitro. Naive dorsal skin constitutively produces H_2S similarly to the concentration produced by the brain. **Conclusion**: We provide the first evidences that H_2S exerted protective effect against acute pruritus mediated via histaminergic and nonhistaminergic pathways in murine dorsal skin, thus making of H_2S donors a potential alternative therapy to treat acute pruritus. **Acknowledgments**: CNPq, CAPES and FAPESP for financial support: Experimental protocols - process number: 100/2013/CEUA

04.011 Anti-inflammatory effect of methyl gallate on experimental arthritis: Inhibition of neutrophil recruitment, production of inflammatory mediators, and activation of macrophages. Correa LB[1,2], Pádua TA[1,2], Seito LN[1], Costa TEMM[1,2], Andrade-Silva M[1,2], Candéa ALP[1,2], Rosas EC[1,2], Henriques MG[1,2] [1]Farmanguinhos-Fiocruz – Farmacologia Aplicada, [2]CDTS-INCT-IDN

Introduction: Arthritis is joint inflammation that can cause edema, pain, and loss of function in the joints. The most common types of arthritis are osteoarthritis, gout and rheumatoid arthritis (RA) (Scott, Lancet 376:1094, 2010). The inflammatory process in RA results from the dysregulation of proinflammatory cytokines together with the infiltration of the polymorphonuclear and mononuclear leukocytes, highlight the crucial role of the immune system in the pathogenesis of RA (Firestein, Nature. 423:356, 2003). Experimental models of arthritis provide an important approach for evaluating potential anti-inflammatory molecules. In this work we studied the effect of methyl gallate (MG) a prevalent phenolic acid in the plant kingdom, that have remarkable biological effects, such as antioxidant (Whang, Exp. Mol. Med. 37:343, 2005), antitumor (Lee, Biochim. Biophys. Acta. 1830:4017, 2013), and antimicrobial (Acharyya, J. Med. Microbiol. 64:901, 2015) activities. Although some indirect evidence suggests anti-inflammatory activity for MG, there are no studies concerning *in vivo* effects of this polyphenol. Herein, we have demonstrated that MG suppresses articular inflammation in the experimental model of zymosan-induced arthritis (ZIA). **Methods:** Mice were pretreated with MG (7 mg/kg 1 hour before the stimulus) and the anti-inflammatory effects were evaluated in murine model of ZIA (500 µg/cavity). Edema formation, leukocytes accumulation and levels of proinflammatory mediators were assessed after MG treatment. The direct effect of MG in neutrophils chemotaxis and adhesion was evaluated by Boyden chamber assay and cell adhesion assay, respectively. The effect of MG on the expression of COX-2 and iNOS in macrophages J774A.1 stimulated with zymosan was measured by immunoblotting. **Results:** Oral administration of MG (7 mg/kg) attenuates ZIA, reducing edema formation (6 h - 23%; 24 h - 49%), leukocyte migration (mainly neutrophils: 6 h - 58%; 24 h - 70%), and the production of inflammatory mediators (IL-1β, IL-6, TNF-α, CXCL-1, LTB_4 and PGE_2). Pretreatment with MG inhibited *in vitro* neutrophil chemotaxis elicited by CXCL-1, as well as the adhesion of these cells to TNF-α-primed endothelial cells. MG also impaired zymosan-stimulated macrophages by inhibiting IL-6 and NO production, COX-2 and iNOS expression, and intracellular calcium mobilization. **Conclusion:** Our results showed that MG presents an anti-inflammatory effect in experimental arthritis by targeting multiple cellular events such as the production of various inflammatory mediators, as well as leukocyte activation and migration. **Supported by**: CNPQ, FAPERJ, CAPES. All protocols were approved by Animal Ethics Committees from Oswaldo Cruz Foundation (registered under the number CEUA LW-43/14).

04.012 Teriflunomide and methotrexate injected intrathecally inhibits LPS-induced knee-joint arthritis in rats. Norões MM, Tonussi CR UFSC – Farmacologia

Introduction: Leflunomide and methotrexate are disease-modifying antirheumatic drugs classically used systemically in the treatment of rheumatoid arthritis due to the inhibition of proliferation of immune cells and cytokines in the joint cavity. Based on these potential inhibitory effects on glial cells, our hypothesis is that the direct administration of these drugs in the spinal cord can reduce the SNC potentiation of the peripheral inflammation. Material and **Methods**: Female Wistar rats, weighing between 200 and 220 g, received intrathecal injections of drugs 2 hours before the articular injection of LPS (30 ng/ 50 µl; i.a.) in the knee joints previously sensitized with carrageenan (300 µg/ 20 µl; i.a.). Inflammatory-induced incapacitation was measured hourly by the paw elevation time (TEP; s) in 1-min period of observation, and edema was evaluated by the articular diameter increase (DA; cm) taken just after each incapacitation measurement. Five hous after LPS stimulus the synovial fluid was collected for the total leukocyte counting (CT; cells/ mm3)(CEUA/UFSC approval number: PP00723). **Results** and Discussion: Intrathecal administration of teriflunomide (active metabolite of leflunomide) (0.1 and 20 µg/ 10 µl) and methotrexate (25 g/ 10 µl) were able to reduce cell migration to the synovial fluid, incapacitation and joint edema induced by LPS, unlike when given by intraperitoneal route, suggesting that the interaction site for antiarthritic effects of drugs was restricted to spinal cord microenvironment. Co-administration with uridine (10 µg) only reversed the inhibitory effects produced by the lower dose of intrathecal teriflunomide, suggesting the higher dose of the drug acted by a mechanism independent from inhibition of nucleotide synthesis. Co-administration of intrathecal teriflunomide (0.1 µg) and bumetanide (60 µg), a blocker NKCC1 cotransporter did not enhance the effects of teriflunomide, suggesting that inhibition of dorsal root reflex and neurogenic inflammation are involved in spinal action of teriflunomide. Intrathecal co-administration of teriflunomide (0,1 µg) and methotrexate (25 µg) caused a summation of their inhibitory effects on inflammation. Conclusion: These data suggest that direct intrathecal methotrexate and leflunomide administration could be a new strategy for the management of rheumatoid arthritis, probably avoiding systemic side effects. Financial Support: CAPES/CNPq.

04.013 Influence of estradiol on the mobilization of leukocytes and serum chemokines release after intestinal ischemia and reperfusion in rats. Fantozzi ET[1], Ricardo-da-Silva FY[2], Rodrigues-Garbin S[1], Vargaftig BB[1], Oliveira-Filho RM[1], Breithaupt-Faloppa AC[2], Tavares-de-Lima W[1] [1]ICB-USP – Farmacologia, [2]FM-USP – Cirurgia

Introduction: Leukocyte mobilization is a hallmark of intestinal ischemia and reperfusion (i-IR) that mediates local and remote organ inflammatory responses. Understanding the mechanisms involving the traffic of leukocytes during i-IR may help to modulate the tissue injury induced by this event. Sex hormones, notably estradiol, exert a protective role and render female resistant to the repercussions of i-IR in comparison to males. However, the role of estradiol on the control of leukocyte mobilization and consequent inflammatory response after i-IR is yet unclear. In the present study, we investigated the involvement of this steroid on local and systemic effects of i-IR in ovariectomized (OVx) female rats. **Methods**: The studies were performed in accordance to IACUC from the ICB/USP. After 7 days of OVx, Wistar rats (60 days old) were submitted to 45 min occlusion of the superior mesenteric artery, followed by 2 h reperfusion. OVx-Sham i-IR rats were used as controls (Sham). i-IR was also induced in a group of rats with intact ovaries. Estradiol (280 mg/kg, s.c.) was given to OVx rats 24 h before induction of i-IR (IR+E). The index (%) of mobilization (IM) of circulating leukocytes was determined quantifying the leukocytes before and after i-IR (hematological analyzer). Neutrophil accumulation into the gut was assessed by myeloperoxidase (MPO) activity assay and, the ability of neutrophil to migrate was quantified by in vitro chemotaxis assay. Bone marrow cells (BMC) count was performed using optical microscopy. Systemic mediators (MIP-2 and CINC-1) were quantified by Multiplex ELISA. Comparisons between groups were made by one-way ANOVA followed by Tukey posttest. **Results**: Ischemia and reperfusion significantly increased blood leukocytes number in rats with intact ovaries (IM: 128.6%) in comparison to sham rats. i-IR did not significantly alter blood leukocyte mobilization in i-IR OVx rats (59.7%) but estradiol treatment significantly increased IM to 90.4%. Blood granulocytes and bone marrow cell count were increased after i-IR in OVx rats in comparison to rats with intact ovaries but this increase was unaffected by estradiol. In contrast, the hormone was able to reduce the increased levels of serum MIP-2 and CINC-1 as well as the elevated MPO activity in the gut of i-IROVx. In vitro neutrophil migration after i-IR was increased cells from OVx rats but not from OVx rats treated with estradiol. **Conclusion**: Our data suggest that during i-IR, estradiol modulates the traffic of circulating leukocytes, especially neutrophils, by a CINC-1 and MIP-2 dependent mechanism and can modulate the MPO activity in gut. Financial Support: CNPq and Fapesp (2013/15291-0). Ethics Committee 111/10/03

04.014 Role of estradiol on leukocyte mobilization and systemic chemokines after intestinal ischemia reperfusion in male rats. Ricardo-da-Silva FY[1], Fantozzi ET[2], Rodrigues-Garbin S[2], Oliveira-Filho RM[2], Vargaftig BB[2], Breithaupt-Faloppa AC[1], Tavares-de-Lima W[2]
[1]FM-USP – Cirurgia Cardiovascular e Patofisiologia da Circulação, [2]ICB-USP – Farmacologia

Introduction: Intestinal ischemia and reperfusion (i-IR) leads to intestinal injury where neutrophils play a pivotal role. Data from literature indicate that trauma-induced neutrophil activation is limited by estradiol. However, the cellular mechanisms underlying the protective effects of estradiol on the intestinal injury remain to be clarified. In this study we focused on the therapeutic effects of estradiol on the leukocyte mobilization and systemic chemokines release after i-IR. **Methods**: The study was performed in accordance to IACUC of the Institute of Biomedical Sciences, University of Sao Paulo. Anesthetized male rats (Wistar, 60 days old) were submitted to superior mesenteric artery occlusion for 45min, followed by 2 h of reperfusion. As controls were used Sham-operated animals (Sham i-IR). Estradiol (17-β) was given (280 mg/kg, i.v.) 30 min after induction of i-IR (E30). Bone marrow cell (BMC) and white blood cell (WBC) counts were assessed. Also, during i-IR in a group of rats the intestine was placed in a plastic bag and intestinal fluids were collected in order to quantify the total and differential leukocyte counts. CINC-1. MIP-1α and MIP-2 (Multiplex) were quantified in serum samples. ICAM-1 expression was determined in the mesenteric vessels (immunohistochemistry). Finally, neutrophils spontaneous migration were evaluated by in vitro assay. Data were analyzed by comparisons between groups using a one-way ANOVA followed by Bonferroni posttest ($p< 0.05$). **Results**: i-IR caused a reduction of BMC count while, estradiol partially reverted the number of BMC count (Sham: 55.46 ± 4.54; i-IR: 26.33 ± 4.57; E30: 39.19 ± 2.04 x 10^6 cells/ml; n= 9-12). i-IR elevated the total WBC counts, notably granulocytes, that was reduced by estradiol treatment (Sham: 12,492 ± 904.3; i-IR: 17,362 ± 1,005; E30: 14,382 ± 384.2 cells/mm^3; n=9-12). The estradiol treatment was effective in reducing the increased leukocyte counts in the intestinal fluid increased after i-IR (Sham: 2,710 ± 0.27; i-IR: 4,689 ± 0.57; E30: 2,729 ± 0.3 cells x 10^3/mL; n=9-10). i-IR increased systemic release of chemokines that were decreased by estradiol treatment (CINC-1: Sham: 203.9 ± 37.74; i-IR: 1,923 ± 388.1; E30: 361.8 ± 57.85 pg/mL; MIP-1α: Sham: 16.55 ± 1.3; i-IR: 159.3 ± 36.38; E30: 56.22 ± 8.35 pg/mL; MIP-2: Sham: <24.05; i-IR: 306.5 ± 123.8; E30: 56.1 ± 12.52 pg/mL; n=9-10). The expression of ICAM-1 increased in i-IR (Sham: 0.067 ± 0.005; i-IR: 0.107 ± 0.008; E30: 0.107 ± 0.006 pg/mL; n=4 with 2 sections per animal and 10 vessels per section). Neutrophil spontaneous in vitro migration was increased after i-IR and estradiol treatment reversed it (Sham: 9.2 ± 2; i-IR: 34.4 ± 7.2; E30: 4.2 ± 2.0 cells x 10^3/mL; n=12). Conclusion: Our data support that the gut leukocyte mobilization induced by i-IR is modulated by estradiol, likely involving the systemic release of chemokines, which could contribute to neutrophils activation. (Ethics committee nº 111/10-03; FAPESP: 2013/15291-0).

04.015 Targeting the sphingosine pathway to resolution of inflammatory response induced by LPS. Perez DA, Athayde RM, Reis AC, Secchim LR, Vago JP, Resende BM, Teixeira MM, Sousa LP, Pinho V UFMG

Introduction: Sphingosine, an important sphingolipid derived from plasma membrane, plays a fundamental role in many cellular processes including cell proliferation, angiogenesis, senescence and apoptosis but its role to inflammation resolution remains unclear. Here, we propose to evaluate the role of sphingosine pathway on neutrophil accumulation at pleural cavity of LPS-challenged mice. **Methods** and **Results**: Mice received i.pl. administration of LPS (250 ng/cavity) or PBS. LPS induced neutrophil recruitment that was increased at 4 h, peaked at 8–24 h, and declined thereafter. The expression of sphingosine-1-phosphate receptors 1, 2 and 3 (S1PR1, S1PR2 and S1PR3) increase after 4h i.pl. LPS injection. Intraperitoneal treatment with sphingosine pathway modulators such as the L-cycloserine, DL-threo-Dihydrosphingosine (DTD), Cay 10444, FTY720, Cay 10621 and JTE013 at 4 h after LPS administration, decreased the number of neutrophil and increased the percentage of apoptotic cells and phagocytosis of apoptotic cells (efferocytosis). *In vitro,* all drugs, also increase human apoptotic neutrophil. Conclusion: We suggest that regulation of sphingosine pathway in vivo may represent a pro-resolving strategy for the treatment of neutrophilic inflammation.

04.016 Lipoxin A4 prevents Malaria-induced Acute Respiratory Distress Syndrome by neutrophil cytoskeletal remodeling impairment. Pádua TA[1], Torres ND[1], Silva JD[2], Costa MFS[1,3], Candéa AP[1], Rocco PRM[2], Souza MC[1], Henriques MG[1,3] [1]Farmanguinhos-Fiocruz – Farmacologia Aplicada, [2]IBCCF-UFRJ – Investigação Pulmonar, [3]CDTS-INCT/IDN-Fiocruz

Introduction: Malaria-induced acute respiratory distress syndrome (M-ARDS) can be triggered locally by parasites components or may be secondary of systemic inflammatory response. Furthermore, it is well established that experimental malaria induced ARDS depends on neutrophil infiltration. Lipoxin A_4 (LXA_4) is an anti-inflammatory eicosanoid, which effect in lung tissue during pulmonary non-infectious ARDS has been described; however, it is not clear if LXA_4 would exerts effects on inflammatory components involved in M-ARDS. Thus we hypothesized that LXA_4 could be regulating pulmonary and/or extrapulmonary inflammatory response preventing M-ARDS. **Methods**: C57Bl/6 mice were infected or not with 10^6 parasitized red blood cell (*P. berghei* (*Pb*) ANKA) intraperitoneally, one hour before, the animals were treated with LXA_4 (0.5 µg/kg/day, 200µl). The treatment was given during 6 days and all experiments were evaluated at the sixth day after the infection. Pulmonary edema was measured by Evan's blue extravasation to the tissues. For the pulmonary mechanical evaluation, the mice were mechanically ventilated with a constant flow ventilator. Airflow and tracheal pressure (Ptr) were measured. In an open chest preparation, Ptr reflects transpulmonary pressure (PL). (ΔP1) and viscoelastic/inhomogeneous (ΔP2) pressures, as well as static elastance (Est), were computed by the end-inflation occlusion method. IL-6, TNF-α, IL-10, CXCL1 and CCL2 were analyzed by ELISA in pulmonary tissue. Neutrophil isolated from femurs were allowed to migrate in Boyden chamber or were stained with rhodamine-phalloidin and analyzed by fluorescence microscopy. **Results**: Pre-treatment with LXA_4 prevents M-ARDS related parameters as lung edema, lung mechanics impairment and myeloperoxidase activity in pulmonary tissue. M-ARDS prevention by LXA_4 pre-treatment was accompanied by decrease in numbers of circulating neutrophils. Therefore, analysis of bone marrow neutrophils revealed that *in vivo* pre-treatment with LXA_4 did not affect neutrophil maturation, apoptosis or chemokine (C-X-C motif) receptor 2 expression. However, bone marrow neutrophils recovered from LXA_4-treated mice were unable to migrate *in vitro.* The *in vitro* treatment with LXA_4 also impaired neutrophils to migrate towards CXC ligand 1 and plasma recovered from *P. berghei* infected mice. Furthermore, we observed that LXA_4 impaired neutrophil cytoskeleton remodeling by inhibiting F-actin polarization. **Conclusion**: LXA_4 prevents malaria induced ARDS by regulating neutrophil egress from bone marrow by a mechanism that involves impairment of cytoskeletal remodeling. **Financial support**: Fiocruz, Faperj, CNPq, CAPES. The animal's procedures were approved by the Committee on Ethical Use of Laboratory Animals of FIOCRUZ (L052/12).

04.017 Inhibition of N-Type voltage-gated calcium channel by toxin from the spider *Phoneutria nigriventer* as a new strategy to control the symptoms and signs of multiple sclerosis. Silva RBM[1], Gomez MV[2], Campos MM[1] [1]INTOX-PUCRS, [2]IEP-UFMG

Introduction: Multiple sclerosis (MS) is an autoimmune demyelinating disorder of the central nervous system, affecting 2.3 million people worldwide (Ransohoff, Nat Rev Neurol, 11, 134, 2015). The disease is orchestrated by infiltration of autoreactive T and B cells, as well as macrophages, and high levels of pro-inflammatory cytokines (Rush, Nat Rev Neurol, 11, 379, 2015). Voltage-gated calcium channels (VGCCs) have been described as pivotal regulators of immune cells and cytokine production (Zamponi, Nat Rev Drug Discov, 15, 19, 2016). We investigated the effects of spinal administration of the N-type VGCC blocker Phα1β, isolated from the spider *P. nigriventer*, in a mouse model of multiple sclerosis. **Methods:** Experimental autoimmune encephalomyelitis (EAE), a classical model of MS, was induced in Female C57/BL6 mice, by s.c. injection of complete Freund's adjuvant oil (200 □l), containing 200 µg MOG_{35-55} peptide and 500 µg *M. tuberculosis* extract H37Ra, into the flank. The animals also received 300 ng of *Pertussis* toxin (i.p) on day 0 and day 2 post-immunization. The neurological impairment was determined using a clinical scale, seven days post-immunization. Tactile and thermal hypersensitivity were evaluated using von Frey filaments (0.4 g, 10 applications, right hindpaw) and the hot-plate test (50 ± 1 °C, 30 s cut-off), respectively, during 25 days. The rotarod test was used to analyze motor coordination (at 16 rpm, maximum time of 60 s), and the spatial memory was assessed using the object location task. Mice were rated on 8-point neurological severity scale to measure the general neurological state. The animals were euthanized at 25 days, and serum, spleen, brain and spinal cord were collected to evaluate pro- and anti-inflammatory cytokines. Ziconotide, a classical N-type VGCC blocker, or Phα1β (25-100 pmol/site) were injected intrathecally (i.t.); fingolimod (0.3 mg/kg), the drug clinically used to treat MS, was administered orally. **Results:** The nociceptive changes elicited by EAE induction were markedly reversed by the Phα1β toxin (47 ± 11%), and partially diminished by ziconotide and fingolimod. Moreover, the i.t. administration of Phα1β or the oral dosage of fingolimod produced a marked decrease of clinical scores and neurological severity (47 ± 6% and 55 ± 11%; 32 ± 4% and 52 ± 8%, respectively), allied to an improvement of mouse locomotor activity and spatial memory test. Ziconotide failed to display any improvement of these parameters. Of note, Phα1β significantly reduced the IFN-γ, IL-17 and TNF production elicited by MOG_{35-55}, whereas it induced a significant increase of the anti-inflammatory cytokine IL-10, in brain and spinal cord. **Discussion:** Present data brings novel evidence indicating that pharmacological modulation of N-type VGCC by the spider toxin Phα1β greatly improves the symptomatic and inflammatory responses in a mouse model of MS. This might represent a promising strategy for managing MS in a near future. **Financial support**: CAPES, Edital 63, Toxinologia; CNPq, PUCRS. Animal Ethics Committee approval: (CEUA-PUCRS, 14/00424).

04.018 Bosentan for the treatment of ulcerative colitis, it really works? Maria-Ferreira D[1], Dallazen JL[1], Góis MB[2], Sant'Ana DMG[2], Rae GA[3], Baggio CH[1], Werner MFP[1] [1]UFPR-Farmacologia, [2]UEM – Biosciences and Pathophysiology, [3]UFSC – Farmacologia

Introduction: Ulcerative colitis (UC) is an increasingly chronic disease that affects the colon with unknown etiology. The conventional therapies for UC have been based on the use of corticosteroids, aminosalicylates, immunomodulators, and antibiotics and despite the available treatment options, most of these therapies have side effects or high cost, leading the search for new alternative therapeutic strategies. Bosentan, a nonselective ETA/ETB receptor antagonist, is primarily used for the treatment of patients with pulmonary hypertension (PAH), and previous studies have demonstrated that this drug ameliorates colonic inflammation in TNBS colitis model in rats. In addition, it has been already proposed that endothelin's (ET-1, 2 and 3) are involved in the pathogenesis of human inflammatory bowel disease (McCartney *et al*., Life Sciences, v. 71, p. 1893–1904, 2002 and Claudino *et al*., Canadian Journal of Physiology and Pharmacology, v. 88 (6), p. 661-7, 2010). Thus, in this study we aimed to investigate the protective effects of Bosentan in the dextran sulfate sodium (DSS)-induced colitis in mice, a widely used model of inflammatory bowel disease. **Methods:** Colitis was induced by administration of DSS for 5 days followed by 2 days of water. The animals were orally treated with vehicle (water, 1 ml/kg) or Bosentan (1, 3 and 10 mg/kg) daily. Colitis characteristics (body weight change, presence of blood in feces and fecal consistency) were monitored daily. In the day 8, the colons were collected and their length was measured. After, samples were homogenized for indirect quantification of neutrophil infiltration (myeloperoxidase, MPO) in the colon tissue. The colonic tissues were also used for histological evaluation. All protocols were performed upon approval by the Committee of Animal Experimentation of the Federal University of Parana (CEUA/BIO – UFPR, 928). **Results and Discussion:** The treatment with Bosentan reduced the colitis score (day 8, 1 mg/kg: 42%; 3 mg/kg: 22% and 10 mg/kg: 47%), and protected mice from weight loss (day 8, 1 mg/kg: 44%; 3 mg/kg: 39% and 10 mg/kg: 63%), reducing the macroscopic damage when compared to the DSS group. Bosentan also prevented the reduction of colon length (1 mg/kg: 8.4 ± 0.5 cm and 10 mg/kg: 8.4 ± 0.3 cm) when compared to DSS group (DSS: 6.6 ± 0.2 cm) and reduced the MPO levels in 34% (3 mg/kg), and 52% (10 mg/kg), when compared to the DSS group (9.2 ± 0.6). However, although Bosentan has significantly improved macroscopic scores and inflammatory parameters, we did not observed histological changes in any parameter analyzed, including presence of goblet and inflammatory cells, cell infiltration in the submucosa, ulceration, destruction of tissue architecture, thickness and flatness of the mucosa and abscesses of the crypts. **Conclusion:** Collectively, the present study demonstrates that the beneficial effect Bosentan in reducing the severity of DSS-induced colitis should be regarded with caution, since the protection observed appears to be mainly because of the reduction of the disease characteristics and inflammatory cell infiltration without changing the histological parameters. Thus, further research is needed concerning Bosentan as a treatment option in UC. **Support:** CNPq (484804/2012-0)

04.019 Hydroquinone exposure contributes to induction and aggravation of experimental arthritis in rats. Heluany CS[1], Kupa LVK[1], Viana MN[2], Fernandes CM[2], Farsky SHP[1] [1]FCF-USP – Análises Clínicas e Toxicológicas, [2]IBu – Farmacologia

Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune disease, which has been associated to cigarette smoking, as a trigger factor in the development of RA or as a worsen agent in pre-existing disease. However, the role of cigarette components on RA induction remains unknown. Hydroquinone (HQ) is a phenolic compound found in high concentrations in cigarette, as well as it is a benzene metabolite. Type II collagen-induced arthritis (CIA) is widely accepted as a valid RA animal model for mimicking human RA. Thus, we aimed to investigate the role of HQ exposure on CIA development in *Wistar* rats and the involved mechanisms. **Methods:** Animals were immunized s.c. at the tail base by using 200 µL of bovine type-II collagen emulsified 1:1 with complete Freund's adjuvant (CFA). A booster injection of 100 µL was administered 7 days later. Rats were exposed to saline or HQ vehicle (saline:ethanol, 20:1) or HQ 25ppm. To evaluate the effects of HQ exposure (1 hour/day, using a nebulization chamber) on CIA, animals were divided in 4 groups according to HQ exposure procedures: A-35 consecutive days, which comprehended one week before collagen injection and 28 days of disease development; B-first 14 days, one week before and 7 days after collagen injection; C-only on the last 7 days of experimental period (28^{th}-35^{th} days); D-first 21 days, one week before and 14 days after collagen injection. Animals of groups A, B and C were sacrified on day 35 and from group D on day 22. Animals were submitted to clinical evaluation (score 0-4: 0=no arthritis; 1-2=weak arthritis, with inflamed digits; 3=medium arthritis, with more than 2 digits and an inflamed footpad; 4=strong arthritis, with all inflamed digits and paws), hematological parameters, histological analysis, quantification of cytokines levels, citrullinated proteins and splenocytes proliferation. n= 4 animals per group. **Results:** Data obtained showed that in groups A and C, HQ exposure elevated scores (3-4) of CIA when compared to HQ vehicle or saline exposure (1-2), caused weight body loss, marked increase on leukocyte numbers into the synovial fluid and neutrophils influx into the synovial membrane, pannus formation and hyperplasia of synovial cells in the synovia. However, HQ exposure in groups A and C did not alter the number of circulating cells and the production of citrullinated peptides in the serum and in the bronchoalveolar lavage. In group B, HQ exposure induced score 2-3, enhanced pannus formation and did not modify other parameters in comparison to saline or vehicle groups. In the sensibilization phase (group D), HQ exposure caused pronounced reduction in the splenocytes proliferation when compared to vehicle or saline exposures, and did not alter the production of cytokines IL-6, IL-1ß or TNF-α. **Conclusion:** Our data show that HQ exposure has a role on CIA disease and that HQ may be a determinant component of cigarette on RA induction and development. As HQ exposure caused more severe CIA when exposed during the entire and at the end period of the disease, and it did not enhance the parameters of the sensibilization phase, we suggest that HQ may act locally on the synovia. Further experiments are being carried out to investigate this hypothesis. **Supported by** Cnpq; FAPESP 2014/07328-4. **Protocol CEUA number 435**.

04.020 Role of ACKR2 in experimental COPD induced by cigarette smoke inhalation. Coutinho DS[1], Ferreira TPT[1], Dias DF[1], Arantes ACS[1], Arantes ACS[1], Ciambarella BT[1], Serra MF[1], Silva PMR[1], Locati M[2], Martins MA[1] [1]Fiocruz – Inflamação, [2]Humanitas Clinical and Research Center – University of Milan

Introduction: ACKR2 is an atypical chemokine receptor implicated in sequestering and internalization of chemokines and resolution of inflammation. Remarkably, ACKR2 is highly expressed on human alveolar macrophages of COPD, however, its role remains elusive. This study assessed the impact of ACKR2 in a murine model of COPD triggered by cigarette smoke (CS) inhalation. We hypothesized that ACKR2 has a protective role on crucial pathologic changes in COPD mice. **Methods**: C57Bl/6 mice were distributed into 4 groups: (1) WT mice exposed to ambient air (AA), (2) WT mice exposed to CS, (3) ACKR2$^{-/-}$ mice exposed to AA and (4) ACKR2$^{-/-}$ mice exposed to CS. Animals were exposed to 3 CS cycles every day, 4 cigarettes per cycle for 12 weeks. Analysis were performed 24 h after the last exposure to CS. **Results:** ACKR2$^{-/-}$ mice showed a significant increase in mean linear intercept (Lm) values both in AA (31.6 ± 0.4 to 35.4 ± 0.7 μm) and CS conditions (41.8 ± 0.5 to 44.5 ± 0.4 μm). ACKR2$^{-/-}$ mice also exhibited a substantial decrease in catalase levels following AA exposure (9.9 ± 0.1 to 1.9 ± 0.1 U/mg ptn), suggesting the existence of a COPD predisposing phenotype. Following CS exposure, lung MPO levels showed a 2-fold increased in ACKR2$^{-/-}$ mice compared to WT mice. Finally, though the levels of IL-17, TNF-α and CCL3 were slightly higher in the lung of ACKR2$^{-/-}$ as compared to WT mice grown in ambient air, levels of these inflammatory mediators were similarly increased in the lung of WT and ACKR2$^{-/-}$ mice exposed to CS. **Conclusion:** These findings suggest that the atypical chemokine receptor ACKR2 prevents the development of pivotal COPD features, including chronic inflammation and emphysema. **Financial support:** *UE FP7- 2007-2013; HEALTH-F4-2011-281608.* **Animal Research Ethical Committee:** *L0-30/15*

04.021 cAMP elevating agents induce resolution of acute inflammation dependent on Annexin A1. Lima KM[1], Negreiros-Lima GL[1], Caux TR[1], Vago JP[1], Tavares LP[1], Aribada RG[1], Carmo AAF, Galvão I, Costa BRC[1], Soriani FM[1], Perretti M[2], Silva PMR[3], Pinho V[1], Solito E[2], Teixeira MM[1], Sousa LP[1] [1]UFMG, [2]Queen Mary University of London, [3]Fiocruz

Introduction: Annexin A1 (AnxA1) is a glucocorticoid-regulated protein endowed with anti-inflammatory and proresolving properties. We previously showed that Rolipram (ROL), a phosphodiesterase-4 inhibitor, induces resolution of acute inflammation and AnxA1 expression. In this study, we investigated the ability of ROL and db-cAMP (cAMP mimetic) to modulate AnxA1 expression and evaluated whether AnxA1 is involved in the pro-resolving ability of these compounds. **Methods:** BALB/c mice were challenged with an intrapleural injection of LPS or PBS and later received an injection of ROL (6 mg/kg/i.p.), db-cAMP (100µg/mouse/i.pl.) or Dexamethasone (DEXA, 2 mg/kg/i.p.). A nonselective AnxA1 receptor antagonist (BOC-1 -5 mg/kg/i.pl.) or a PKA inhibitor H89 (100µg/kg/i.pl.) were given before the drugs. The cells from the pleural cavity were harvested after treatments and processed for total and differential leukocyte counts, apoptosis and western blot analysis for AnxA1. *In vitro* studies were carried out in PMA-differentiated THP-1 cells (a human monocytoid cell line), which were treated with ROL and db-cAMP at different times and concentrations. To investigate whether the effect of Rolipram was dependent of PKA pathway, we used two PKA inhibitors: H89 and Rp. Human neutrophils were also treated with ROL e db-cAMP to analyze the ability of these drugs to induce *in vitro* apoptosis. **Results:** The ROL and db-cAMP treatment shortened resolution intervals, improved resolution indices and increased AnxA1 expression. *In vitro* studies showed that ROL or db-cAMP induced AnxA1 expression and phosphorylation and this effect was prevented by PKA inhibitors, suggesting the involvement of PKA on ROL-induced AnxA1 expression. Moreover, ROL and db-cAMP induced AnxA1-dependent neutrophil apoptosis *in vitro* suggesting a functional involvement of the endogenous AnxA1. Akin to these *in vitro* findings, H89 prevented ROL and db-cAMP-induced resolution of inflammation, and it was associated with decreased levels of intact AnxA1. Interestingly, BOC1 also prevented ROL and db-cAMP-induced resolution and the neutralization of AnxA1 resulted in loss of the proresolving effects of these agents. **Conclusion:** Our results showed that AnxA1 is at least one of the endogenous determinants mediating the pro-resolving properties of cAMP elevating agents. **Financial support:** CNPq, FAPEMIG and CAPES. **Research approval Animal Ethical Committee:** CETEA/UFMG, Protocol number 15/2011.

04.022 Potential pro-resolutive effects of rolipram on pathogenesis of chronic nephropathy induced by doxorubicin. Costa WC[1], Silva JD[1], Barroso LV[1], Campolina GH[1], Reis AC[1], Braz GGS[1], Santos APB[1], Pinho V[1] [1]UFMG – Morfologia

The chronic inflammation has been associated to an ineffective resolution of inflammation response. Thus, the development of therapy based in induction or activation of inflammation resolution program must be an innovative approaches to treatment of unresolved inflammation. In this study, we have been investigating the effects of treatment with rolipram, a selective PDE4 inhibitor with putative pro-resolutive actions, on pathogenesis of chronic nephropathy induced by doxorubicin. The nephropathy was induced by single dose of doxorubicin (10mg/kg) in the tail vein of Balb/c mice. All experimental animals injected with doxorubicin developed nephropathy that was maximal at day 14 after injection. To verify the effects of rolipram, doxorrubicin-injected mice were daily treated with rolipram (6.0 mg/kg) from day 7, when histological changes and renal injury has already been established, to day 14 after exposure to doxorrubicin. Control mice received vehicle dose (PBS+DMSO) at the same period that was administered the treatment with rolipram. Treatment with rolipram induced recorery in serum total protein and albumin and reduced weight loss and prostration of mice. In addition, rolipram decreased glycoproteins accumulation in glomerulus and renal tubules and ameliorate histophatological renal damage. Taken together, this preliminary study provides evidence that rolipram may have pro-resolutive effects in established chronic inflammation during nephropathy induced by doxorubicin. Financial Support: CAPES/FAPEMIG. Ethics Committee UFMG: 263/2015

04.023 Ouabain inhibits neutrophil migration through downregulation of p38 MAPK activation. Cavalcante-Silva LHA, Lima EA, Galvão JGFM, Costa JOM, Freitas JAM, Rodrigues-Mascarenhas S UFPB

Introduction: Ouabain, a Na^+,K^+-ATPase inhibitor, was first identified as an endogenous substance (Hamlyn, JM; Proc Natl Acad Sci USA; 88:6259, 1991). Recently, ouabain was shown to affect various immunological processes (Rodrigues-Mascarenhas, S; Ann NY Acad Sci; 1153:153; 2009), including inflammation (Leite, JA; Mediators Inflamm; 2015:1; 2015), but little is known about the mechanisms involved in its anti-inflammatory effect. Thus, the aim of this work was to evaluate ouabain mechanism of action on neutrophil migration. **Methods**: In this work, female Swiss albino mice (2 months old) were obtained from Thomas George animal house of UFPB. Firstly, neutrophils obtained from mice peritoneum after thioglycolate broth 4% challenge were submitted to *in vitro* transmigration assay. Chemotaxis was measured by migration through a polycarbonate filter of 5 µm pore size in 24-well transwell chambers. DMEM containing 0.5% FCS plus N-Formyl-Met-Leu-Phe (fMLP) (100 nM), or medium alone as a control for spontaneous migration, was added to the lower chambers. 1.5 x 10^6 cells were added to the upper chambers in absence or presence of ouabain (1 nM, 10 nM, 100 nM, and 1 µM), and were incubated with 5% CO_2 atmosphere at 37 °C. After 4 h, migration was defined by counting the cells that migrated to the lower chambers by optical microscopy. In order to perform *in vivo* experiments, 0.56 mg/kg ouabain or phosphate buffered saline (PBS) was given intraperitoneally (i.p.) for three consecutive days. Peritoneal inflammation was induced by zymosan (2 mg/mL, 500 µL, i.p.) one hour after the last injection on day 3. The cells were isolated 4h after challenge, counted by optical microscopy, and then submitted to intracellular staining protocol of P-p38, and analyzed by flow cytometry. Statistical analysis was performed using ANOVA one way followed by Turkey test. **Results**: fMLP stimulus increased neutrophil migration around 70% ($p < 0.01$) when compared to spontaneous migration. On the other hand, when neutrophils were treated with ouabain (1 nM, 10 nM, 100 nM, and 1 µM), and stimulated with fMLP, it was observed a reduction of neutrophil migration by 55.4%, 49.1%, 60%, and 61.1% ($p < 0.01$), respectively, when compared to fMLP alone. Besides that, zymosan challenge increased cell influx by 85% when compared to control group (PBS). When animals were treated with ouabain, it was observed 47.5% cell migration reduction when compared to zymosan group. It is noteworthy that ouabain treatment alone did not modulate peritoneum cell number. Lastly, neutrophils recovered from zymosan presented 65% P-p38 levels increase when compared to control group cells ($p < 0.01$); whereas neutrophils recovered from animals treated with ouabain and challenge by zymosan presented 44.1% P-p38 levels reduction ($p < 0.01$). Also, ouabain treatment alone did not modulate P-p38 levels. Conclusion: Taken together, these data support *in vitro* and *in vivo* ouabain inhibitory effect on neutrophil migration. This effect could be related to P-p38 MAPK inhibition, a cell signaling protein involved in neutrophil migration. **Financial support**: This work was supported by CNPq (grant nº 478536/2013-5). Ethical committee number 039/2015.

04.024 Coadjuvant action of Annexin A1 on angiogenesis: potential application to heterologous transplantation. Mimura KKO, Drewes CC, Lacerda JZ, Zanon CF, Greco R, Ansari T, Gil CD, Greco KV, Oliani SM, Farsky SHP FCF-USP

Introduction: The anti-inflammatory protein annexin A1 (ANXA1) confers regulatory functions on vascular development; however, its mechanisms of action and outcomes are still controversial. Here we investigated the role of endogenous ANXA1 in vascular endothelial growth factor-A (VEGF-A)-induced angiogenesis, and the effect of an ANXA1 mimetic (peptide $ANXA1_{2-26}$) on endothelial cell functions and *in vivo* angiogenesis during heterologous transplantation. **Methods:** Human umbilical vein endothelial cells (HUVEC) were treated with $ANXA1_{2-26}$ (1-30μM) or with VEGF-A (50ng/mL) or with both simultaneously, and employed to evaluate cell cycle, proliferation and adhesion molecules expression by flow cytometry; adherence, migration and tube formation on matrigel; F-actin staining by confocal microscopy and ultrastructural immunocytochemical to label ANXA1. Wild-type (WT) or Annexin A1 knockout ($AnxA1^{-/-}$) Balb-C mice were topically treated with $ANXA1_{2-26}$ peptide (1 mg/kg;·10μL)] and/or VEGF-A (10ng/10μL) to investigate the angiogenesis on the subcutaneous tissue by intravital microscopy; and WT mice were treated with $ANXA1_{2-26}$ peptide (100μg/mouse·day) during a heterologous transplantation. **Results**: $ANXA1_{2-26}$ treatment enhanced cell proliferation, migration and actin polymerization in HUVECs, similarly to those evoked by VEGF-A. Differently, $ANXA1_{2-26}$ treatment impaired HUVEC adhesion to matrigel, and induced tubulogenesis only when coapplied with VEGF-A. Angiogenesis in the dorsal subcutaneous tissue of BALB/c mice (WT) was increased following treatment with $ANXA1_{2-26}$ or VEGF-A; nevertheless, basal or VEGF-A-stimulated angiogenesis was equivalent in WT and ANXA1 null mice. From a therapeutic perspective, heterologous transplantation of skin scaffolds was improved in mice treated with $ANXA1_{2-26}$ by inducing angiogenesis, cell influx into allograft tissue and impaired and augmented expression of pro- and anti-inflammatory cytokines, respectively. **Conclusions:** Collectively, our data indicate that VEGF-A-induced angiogenesis occurs in the absence of AnxA1; nevertheless, $ANXA1_{2-26}$ therapy favours angiogenesis and tissue regeneration after transplantation, providing evidence for a potential therapeutic application. **Financial Support:** FAPESP grant 2014/07328-4 Ethics Committee in Animal Experimentation of São Paulo State University of São José do Rio Preto (Nº. 074/2013 and 065/2012).

04.025 Participation of 5-LO pathway in development of mouse model of acute graft-versus-host disease: potential new therapeutic target for GVHD. Rezende BM[1], Bernardes PT[1], Athayde RM[1], Resende CB[1], Gonçalves WA[1], Perez DA[1], Esper L[2], Cisalpino D[3], Cunha TM[4], Castor MGM[5], Machado FS[2], Teixeira MM[2], Pinho V[1] [1]ICB-UFMG – Morfologia, [2]ICB-UFMG – Bioquímica e Imunologia, [3]ICB-UFMG – Microbiologia, [4]FMRP-USP – Farmacologia, [5]ICB-UFMG – Farmacologia

Introduction: Graft-versus-host disease (GVHD) remains a major limitation for bone marrow transplant. 5-LO is an enzyme associated with leukotriene B4 production (LTB4). LTB4 has been associated with intestinal GVHD in humans, however its mechanism of action remain unclear. Objective: We evaluated the participation of the 5-LO/LTB4 axis in GVHD pathogenesis. **Methods**: GVHD was induced in B6D2F1 mice by transplant of 1x107 bone marrow cells + 3x107 splenocytes from SV129 or of 5LO-/- leukocytes. After transplant, intestinal 5-LO mRNA expression was assessed by PCR. To evaluate the effect of pharmacological inhibition of 5-LO, transplanted mice were treated with zileuton (30 mg/kg, 12 h/12 h) by gavage. The liver and intestine were subjected to histopathological analysis, ELISA, NAG and flow cytometry. The levels of LTB4 were evaluated in the blood, liver and intestine by enzymatic immune assay. To evaluate chimerism, the frequency of H2D+H2B+ cells (B6D2F1 cell marker) and H2D+ cells (C57BL/6 and SV129 cell marker) was assessed in the spleen and bone marrow of all groups. 5-LO-/- leukocytes transplantation or zileuton treatment ameliorated GVHD by reducing intestinal and liver injury and serum and hepatic LTB4 levels. These improvements were associated with inhibition of macrophages and CD8+ cells and decreased levels of cytokines and chemokines (IFN-y, TNF-a, IL17, IL12, CCL2, CCL3, CCL5). Transplant of 5-LO-/- leukocytes or zileuton treatment also prolonged survival and reduced GVHD clinical scores. Moreover, transplant of 5-LO-/- leukocytes or zileuton treatment did not interfere in the incidence of chimerism. Conclusion: the 5-LO/LTB4 axis orchestrates GVHD development, revealing a potential new pharmaceutical strategy for repositioning zileuton for the treatment of GVHD in bone marrow-transplanted patients. Number of approval ethics committee (CETEA/UFMG): 135/13. **Financial support**: CAPES, FAPEMIG e CNPq.

04.026 Translocator Protein 18 kDa (TSPO): A Promising Target for Meta-Inflammation. Barioni ED[1], Rocha GHO[1], Oliveira EM[2], Campa A[1], Farsky SHP[1] [1]USP – Análises Clínicas e Toxicológicas, [2]University of Cambridge – Cambridge – Institute of Metabolic Science

Introduction: Obesity is a chronic disease associated to low intensity chronic inflammation, characterized by increased of macrophages influx, adipose cells necrosis and proinflammatory adipokines secretion, such as TNF by adipose tissue inflamed. Translocator protein 18 kDa (TSPO), previously known as the peripheral benzodiazepine receptor (PBR), is expressed in many cell types and considered a new marker of inflammation. TSPO is expressed on adipocytes and has been implicated in many important physiological functions, including adipogenesis, glucose homeostasis, cholesterol transport and steroidogenesis, cellular respiration and immunomodulation. However, effects of TSPO gene and protein expression induced by diazepam, a central and peripheral benzodiazepine receptor agonist, on adipocytes adipogenesis and inflammation is not established. Hence, we here first proposed to investigate the TSPO expression on adipocytes in different conditions. **Methods:** TSPO gene expression on the differentiation process of 3T3-L1 lineage was quantified by Real Time-PCR. The viability of 3T3-L1 pre-adipocytes treated with diazepam (5, 10 and 20 µM), vehicle (DMSO 0,02%) or/and TNF (5 and 10 ng/mL) was quantified by flow cytometer. TSPO gene and protein expression on the differentiation process of 3T3-L1 lineage treated with diazepam, vehicle or/and TNF was quantified by Real Time-PCR and Western Blot, respectively, and 3T3-L1 triacylglycerol storage was quantified by Oil Red staining and free-glycerol colorimetric assay.
Results: Our results showed the positive regulation of TSPO complementary tapes on 3T3-L1 differentiation process. Furthermore, diazepam (5 and 10 µM) or TNF (10 ng/mL) treatment increased and decreased TSPO gene and protein expression, respectively, and did not evoke significantly changes in the viability of 3T3-L1 pre-adipocytes. Finally, 3T3-L1 triacylglycerol storage was not modified by diazepam. **Conclusion:** Our data show that TSPO is expressed on 3T3-L1 cells during the differentiation process, and it is modulated by diazepam, a TSPO agonist, and by TNF. Further studies are being carried out to investigate the role of TSPO activation by different agonist on adipogenesis and meta-inflammation process. **Supported by** FAPESP (2013/11027-7; 2014/07328-4).

04.027 Antagonism of TRPC4/TRPC5 channels increases the severity and mortality of sepsis in mice. Pereira DMS[1], Mendes SJF[1], Castro Jr JAA[1], Aubdool AA[2], Alawi KM[2], Thakore P[2], Grisotto MAG[1], Brain S[2], Fernandes ES[1,2] [1]Ceuma, [2]King's College – Cardiovascular Division

Introduction: Transient receptor potential channels are non-selective Ca^{+2} channels expressed on neuronal and non-neuronal cells, known to play a plethora of roles including in inflammation. TRP Vanilloid 1 (TRPV1) and Ankyrin 1 (TRPA1) are oxidative stress sensors and modulate the systemic inflammatory response (SIRS) to lipopolyssacharide (LPS) as their activation is linked to a protective state in this syndrome (Fernandes et al., J Immunol, 188:5741, 2012; Mendes et al., Int Immunopharmacol, 34:60, 2015). The TRP Canonical 5 (TRPC5) channel was recently suggested as an oxidative stress sensor (Takahashi and Mori, Front Pharmacol, 2:58; 2011), however, little is known of its role in sepsis. Interestingly, TRPC5 can be expressed as homodimers and heterodimers with other TRPC channels such as TRPC4. Here, we evaluated the effects of a selective TRPC4/TRPC5 antagonist in a mouse model of SIRS caused by LPS. **Methods:** Male and female C57BL/6 mice (3-months old) were subcutaneously treated with either vehicle (3% DMSO in saline) or the selective TRPC4/TRPC5 antagonist ML204 (1 mg/kg) twice daily for 5 days. SIRS was then induced by LPS (11.25 million EU/kg, *E. coli* serotype 0111:B4). Vehicle (PBS)-treated animals were used as controls. Temperature changes were measured 5 days following ML204 treatment and over 72h after LPS injection, and were compared with baseline recordings. Disease severity and survival rate were evaluated over 72h following LPS injection. For evaluation of severity, a score was attributed to each of the following parameters as previously described by Mendes *et al.* (2015): grooming behaviour (1-normal grooming, 2-reduced grooming, 3- no grooming), mobility (1-normal mobility, 2-partial impairment, 3-poor mobility, 4-no mobility), piloerection (1- absence, 2- presence) and whipping eyes (1- absence, 2- presence). The summation of the scores attributed to each of the parameters for each animal was taken as severity score index. For comparison, baseline scores were taken for all groups of mice. Additionally, peritoneal lavage cytokine (IL-1β, TNFα and IL-6) levels were determined by cytometric bead array, in samples collected 24h after LPS-injection. **Results:** TRPC4/TRPC5 blockade worsened sepsis severity and induced mortality in 15% of the mice. Also, ML204-treated mice with sepsis (24h) exhibited increased hypothermia ($3^{o}C$ lower) and reduced levels of peritoneal IL-6 (95%) in comparison with vehicle controls. **Conclusion:** Collectively, our data show that TRPC4/TRPC5 channels may play a protective role in sepsis by regulating IL-6 production and thus, influencing temperature regulation, survival and sepsis severity in LPS-injected mice. This is a novel study and thus, other pathways may be involved in TRPC4/TRPC5 protection to sepsis and this remains to be investigated. **Financial support:** FAPEMA, CNPq and CAPES All procedures were approved by the Ethics Committee of UNICEUMA (protocol number 108/14) and carried out in accordance with the Brazilian Society for Animal Welfare.

04.028 Bacterial thioredoxin effects on cytokine production are exacerbated in TRPC5 KO mice with LPS-induced sepsis. Mendes SJF[1], Pereira DMS[1], Silva BLR[1], Aubdool AA[2], Alawi K[2], Thakore P[2], Grisotto MAG[2], Brain SD[2], Fernandes ES[1,2] [1]Ceuma, [2]King's College London – Cardiovascular Division

Introduction: Sepsis is a potentially fatal condition and affects thousands of people annually (Rittirsch et al., 2007). Oxidative stress plays a key role in sepsis, regulating the production of inflammatory mediators, phagocytosis and lysis of the pathogen (Victor et al., 2004; 2005). Thioredoxin (TRX) is a highly conserved protein across species, being produced by all forms of life, from bacteria to humans (Lee et al., 2012). However, despite of having been identified decades ago, its role in sepsis remains unclear. TRX can be reduced during inflammation and in its reduced form, it was shown to activate the Transient Receptor Potential Canonical 5 (TRPC5) channel, a nonselective Ca^{+2} channel found to be expressed on non-neuronal cells (Sossey-Alaoui et al., 1999). Herein, we evaluated the effects of *E. coli*-derived TRX in a mouse model of lipolyssacharide (LPS)-induced sepsis in TRPC5 WT and knockout (TRPC5 KO) mice. **Methods:** Male 129Si/SvImJ WT and TRPC5 KO mice (3-months old) received either vehicle (PBS; 10 ml/kg) or bacterial TRX (20 µg/200 µl) subcutaneously, twice daily for 3 days and then received an intraperitoneal injection of LPS (11.25 million EU/kg, *E. coli*, serotype 111:B4). Vehicle-treated mice were used as controls. Mice were culled 18h following LPS-injection and the peritoneal lavage was collected for analysis. Total and differential cell were evaluated by microscopy and cytokine levels were quantified by using an MSD multiplex array. **Results:** No differences were observed in total peritoneal cell counts when comparing vehicle- and LPS-injected mice of both genotypes. However, TRX treatment caused reduction in the number of total and mononuclear peritoneal cells in LPS-treated WT mice (36%). On the hand, TRX evoked a 2.3-fold increase in the number of total and mononuclear peritoneal cells in TRPC5 KO animals when compared with their LPS-injected controls. LPS-injection increased the peritoneal levels of TNFα and IL-6 in both WT (1.8- and 7.2-fold increase, respectively) and TRPC5 KO mice (1.6- and 3.7-fold increase, respectively). IL-1β was also augmented in WT (5.4-fold increase) but not in TRPC5 KO samples. Whilst treatment with bacterial TRX did not affect cytokine production in WT mice with SIRS, this protein further enhanced the peritoneal levels of TNFα (1.2-fold increase), IL-1β (2.1-fold increase) and IL-6 (2.7-fold increase) in TRPC5 KO-injected mice. **Conclusion:** Collectively, our data show that in the absence of TRPC5, bacterial TRX induces an exacerbated pro-inflammatory response in septic mice; suggesting this receptor may play a protective role in this syndrome. **Financial support:** This research was funded by FAPEMA, CNPq and CAPES. All procedures were conducted in accordance with the UK HO Animals (Scientific Procedures) Act of 1986 and local King's College London ethics approval (HOLC I417F8A77). Lee, S, Kim. Antioxid Redox Signal. Vol 1: 4322; 2012. Rittirsch. J Leukoc Biol, Vol 81: 137; 2007 Sossey-Alaoui, K. Genomics Vol 60: 330; 1999 Victor, VM, Rocha. Int Immunopharmacol. Vol 4: 327; 2004.

04.029 Anti- inflammatory activity of serotonin amide in the coffee beans. Amorim JL[1], Moreira IGS[2], Rezende CM[2], Fernandes PD[1] [1]UFRJ – Farmacologia, [2]UFRJ – Química

Introduction: Coffee is among the most popular drinks in the world and the serotonin amide *N*-behenoil-5-hidroxitriptamide *(*C22-5HT) is present in the surface wax of coffee beans. The simple amides of fatty acids have indicated anti-inflammatory effect, specifically serotonin amides, which have been shown anti-inflammatory activity by inhibiting the expression of caspases. Thus, the aim of this work was to evaluate the anti-inflammatory effect of C22-5HT in the model of formalin-induced paw licking, carrageenan-induced inflammation into the subcutaneous air pouch (SAP), production of reactive oxygen species (ROS) by PMA-stimulated leukocytes and MTT cell viability assay. **Methods:** C22-5HT present in the Arabica coffee beans (*Coffea arabica* L., Rubiaceae) was synthesized by the group of Professor Claudia Rezende, UFRJ. Female *Webster* mice received oral administration of C22-5HT (0.1,1,3 or 10 mg/kg) and evaluated (20-25g; n=6-8) in models of formalin-induced paw licking and leukocyte migration induced by carrageenan into de subcutaneous air pouch (SAP). Mice received oral administration of morphine, acetylsalicylic acid (ASA) or C22-5HT (0.1,1,3 or 10 mg/kg) 1h before injection of 20 µL of formalin (2.5% v/v) into the dorsal surface of the left hind paw. The time that the animal spent licking the injected paw was immediately recorded. The second model used was carrageenan injection into the SAP. After 24h mice were euthanized and the exsudate from SAP were collected for measurements of cell count, protein, nitric oxide (NO), and cytokines. For the evaluation of ROS, leukocytes from the SAP were collected and incubated with C22-5HT (0.1,1 or 3 µM) for 1 hour. After, the cells were treated and incubated with 10 nM PMA and 2 mM DCF-DA. The fluorescence was captured in the FL-1 channel flow cytometer and was expressed as DCF-DA fluorescence. For the evaluation cell viability RAW 264.7 mouse macrophages were incubated with C22-5HT (0.1,1 or 3 µM) for 3, 24 or 48 hours. Statistical significance between groups was determined by ANOVA followed by Bonferroni´s test (*p<0.05). **Results:** C22-5HT showed significant anti-inflammatory activity with reduction on cell migration (vehicle 60.8 ± 8.3x10^6cells/mL *versus* 61.12 ± 9.5x10^6cells/mL, 31.16 ± 8.6*x10^6cells/mL, 39.8 ± 7.7*x10^6cells/mL, and 29.6 ± 8.7* x10^6cells/mL) and protein extravasated (vehicle=264.3 ± 43.4µg/mL *versus* 204.4 ± 42.3µg/mL, 86.6 ± 16.7*µg/mL, 143.1 ± 31.6*µg/mL, and 100.6 ± 28.5*µg/mL) with the doses of 0.1,1,3, and 10 mg/kg. It was also observed reduction in levels of NO produced in all doses tested (vehicle=141.2 ± 31.8µM *versus* 66.7 ± 17.3*µM, 63.1 ± 16.1*µM, 56.2 ± 16.5*µM and 50.1 ± 17.8*µM) and in cytokines levels: INF-γ (vehicle=122.4 ± 38.6pg/mL *versus* 87.7 ± 18.8pg/mL, 82.1 ± 16.2pg/mL, 20.4 ± 7.3*pg/mL, 38 ± 14.8*pg/mL) and TNF-α (vehicle=205.9 ± 31.2pg/mL *versus* 163.3 ± 30.9pg/mL, 86.1 ± 28.6*pg/mL, 48.8 ± 18.6*pg/mL, 9.4 ± 3.8*pg/mL). C22-5HT significantly reduced the production of ROS by PMA-stimulated leukocytes in all concentrations tested (vehicle=197,237.4 ± 8,738 DCF-DA fluorescence *versus* 87,951.6 ± 11,564*DCF-DA fluorescence, 98,852.1 ± 10,391.2* DCF-DA fluorescence, 77,932.3 ± 6,324.1*DCF-DA fluorescence) and did not reduce the cell viability of RAW 264.7 macrophages. **Conclusion:** Initial results demonstrate that C22-5HT produces anti-inflammatory effect, by reducing cell migration, protein extravasation, NO, cytokines production and ROS reduction. The mechanism(s) by which the C22-5HT produces this effect are still under investigation. **Financial support:** CNPq and FAPERJ. **Number of the Committee:** DFBCICB015-04/16.

04.030 Evaluation of anti-inflammatory effect of *Tibouchina granulosa* leaves. Sobrinho AP[1], Ferreira LLC[2], Fernandes PD[1] [1]UFRJ – Farmacologia e Química Medicinal, [2]IVB – Fitoterápicos

Introduction: *Tibouchina granulosa* (Tg) is an ornamental plant popularly named quaresmeira. Personal observation suggests that the aqueous extract (AE) of the leaves given orally has an anti-inflammatory effect in rodents. Our aim was to evaluate the anti-inflammatory effects of AE of the leaves in animal models. **Methods:** Tg leaves were collected in Cachoeiras de Macacu/RJ.A voucher specimen is deposited in the herbarium of IB/UFRJ and received the number 37.931. Infusions were prepared, lyophilized and stored at -20^{0}C until use. Mice female 30-35 g, n=10-15 were used. The experimental model used was carrageenan-induced inflammation into the subcutaneous air pouch (SAP). Animals received 1, 3, 10, 30 or 100 mg/kg, orally 60 minutes before carrageenan injection into the pouch. Mice were euthanized 24 h later and the exudate was collected for measurements of protein, nitric oxide (NO) and cytokines. For the evaluation of reactive oxygen species production (ROS), leukocytes from the SAP were collected and incubated with 1, 10, 30 ug/ml of Tg for 1h. After the cells were treated and incubated with 10 nM phorbol myristate acetate (PMA) and 2'-7'diclorodihidrofluoescein diacetate- DCF-DA. The emitted fluorescence was captured in the FL-1channel flow cytometer and were expressed as DCF-DA fluorescence. Protocol for use of animal's # DFBCICB015-04/16. Statistical significance between groups were determined by ANOVA followed by Newman-Keuls (*p<0.05) **Results:** Reduction in leukocytes migration in SAP model (carrageenan group=64,8 ± 10,7x10^{6}cells/µL control without carrageenan 22,1 ± 3,9x10^{6}cells/µL) In animals pretreated orally and receiving injection of carrageenan into cavity: 32,6 ± 14,4x10^6*cells/µL,32,8 ± 15,0x10^6*cells/µL, 24,6 ± 5,5x10^6*cells/µL 32,1 ± 9,4x10^6*cells/µL,34,7 ± 14,0x10^6*cells/µL 1,10,30 and 100 mg/kg, respectively. Protein measurement: carrageenan group=233,3 ± 74,0µ/mL control without carrageenan 42,7 ± 23,3µL,149,4 ± 138,8µL, 221,2 ± 117,2µL,157,1 ± 65,6*µL, 127,6 ± 35,0*µL,102,4 ± 55,9*µL. NO measurements: carrageenan group=226,9 ± 85,5µ/M control without carrageenan 12,1 ± 4,5µ/M,78,0 ± 27,8*µ/M,81,4 ± 18,2*µ/M, 65,8 ± 33.6* µ/M,53,4 ± 27,25*µ/M e 47,2 ± 37,2* µ/M. Cytokines in treated groups - TNF-α: carrageenan group=260 ± 64,4pg/mL control without carrageenan= 16,3 ± 8,6pg/mL,146,5 ± 110,92*pg/mL, 242,6 ± 68,86pg/mL, 230,1 ± 75,9 pg/mL, 170,7 ± 55,1*pg/mL, 182,0 ± 51,0*pg/mL) and IL-10 (carrageenan group=41,0 ± 4,5pg/mL control without carrageenan 15,6 ± 3,6pg/mL,34,8 ± 25,6pg/mL,22,5 ± 8,2*pg/mL, 23,5 ± 18,2*pg/mL,25,4 ± 14,3* pg/m/L, 54,6 ± 28,8 pg/mL). ROS production in leukocytes stimulated by PMA: PMA-stimulated=65611 ± 15340DCF-DA fluorescence unstimulated=359451 ± 66513DCF-DA fluorescence, 60618 ± 23528*DCF-DA fluorescence, 36516 ± 13259*DCF-DA fluorescence and 79371 ± 37905*DCF-DA fluorescence) at concentrations 1, 10 and 30 µg/mL. **Conclusion:** The results indicate that Tg has anti-inflammatory effect as reduced cell migration, protein extravasation and the production of TNF-α and IL-10 cytokines. Evidenced reduction of ROS production, which is an indicator of intracellular amplification inflammatory response. **Financial support: CNPq, FAPERJ, IVB. Technical support:** Alan Minho.

04.031 Gedunin modulates LPS-induced astrocyte activation. Costa TEMM[1,2], Seito LN[1], Henriques MG[1,2,3], Penido C[1,2] [1]Farmanguinhos-Fiocruz – Farmacologia Aplicada, [2]CDTS-INCT-Fiocruz, [3]INCT-IDN

Introduction: Neuroinflammation is a protective response of the central nervous system (CNS) against infectious insults or injury (Zhang et al, 2015). Astrocytes are key cells involved in neuroinflammation, through the secretion of pro-inflammatory cytokines, growth factors and purines (Be Haim et al, 2015). However, exacerbated activation of astrocytes can be detrimental to the host (Buffo et al, 2010). Gedunin, a natural limonoid from Meliaceae species, has been described as an anti-tumoral and anti-inflammatory compound in different *in vitro* and *in vivo* models; however its role in the CNS is still not kown. Gedunin mechanism of action relies on the inhibition of heat shock protein 90 (HSP90) (Patwardhan et al, 2013) and our group has recently shown that gedunin also binds to myeloid differentiation-2 (MD2) molecule, impairing toll-like receptor 4 (TLR4) signaling pathway (Borges et al, 2015). In this context, the present work aims to evaluate the effect of gedunin on the astrocytic cell line GL261 inflammatory response induced by bacterial endotoxin (lipopolysaccharide, LPS) *in vitro*. **Methods**: GL261 cell line ($2x10^5$/well) were seeded on 24 well plate and treated with gedunin (100 - 0.1µM), with the selective HSP90 inhibitor 17-N-allylamino-17-demethoxygeldanamycin (17-AAG, 1µM) or with dexamethasone (100nM) for 1h. Cells were subsequently stimulated with LPS (1µg/mL) plus Interferon-Y (IFN-Y, 25U/mL) for 6 or 24h. Nitric oxide (NO), tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 levels were evaluated in the supernatant, by Griess and ELISA, respectively. Ciclooxigenase-2 (COX-2), heme-oxygenase-1 (HO-1) and HSP70 expression were evaluated in cell lysates by western blot. Cytotoxic effect of gedunin incubation on GL261 was measured by resazurin salt reduction method. **Results**: Gedunin pretreatment of GL261 cells impaired LPS-induced expression of COX-2, HO-1 and HSP70, in a dose response manner within 6h. Gedunin also reduced the production of TNF-α and IL-6 by GL261 cells 24h after LPS-stimulation. Of note, gedunin incubation did not induce cytotoxic effect on GL261 cells from 100 to 0.1µM. **Conclusion**: Our results demonstrate that gedunin modulates LPS-induced astrocyte activation by triggering increased expression of anti-inflammatory mediators and stress-related proteins, as well as reducing the production of proinflammatory mediators. Gedunin effects might be due to both HSP90 modulation and MD-2 binding, impairing LPS-induced TLR4/MD-2 signaling in astrocytes. References: - Zhang et al, Neuropsychiatr Dis Treat, 11:243, 2015. - Ben Haim et al, Front. Cell. Neurosci. 9:278 ,2015. - Buffo et al., Biochemical Pharmacology 79:77, 2010. - Borges et al, Mol Pharmacol 88:949, 2015. - Patwardhan et al, J. Biol. Chem. 288: 7313, 2013.

04.032 Nimesulide attenuates pentylenetetrazol-induced seizures and increases IL-10 levels in the cerebral cortex and hippocampus. Temp FR, Marafiga JR, Jesse AC, Duarte T, Milanesi LH, Hessel AT, Londero AL, Mello CF UFSM – Farmacologia

Introduction: Inflammation, as part of the innate immune response, is commonly described as sequential events triggered by the activation of pattern-recognition receptors by pathogen- and damage-associated molecular patterns, such as the Toll-like receptors (TLRs). The activation TLR results in the induction of transcriptional factors such as nuclear factor *κ*B, which has the ability to trigger various pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α, COX-2 and thus perpetuate inflammatory reactions in periphery and brain (1,2,3). It is known that seizures increase prostaglandin (PGs) and cytokine levels in the brain (4,5,6), and that PGs are major lipid mediators produced by COX activity. However, it has not been addressed whether an COX-2 inhibitor, such as nimesulide, decreases cytokine production and whether such an effect is associated with seizure suppression. Therefore, in this study we investigated whether the subchronic administration of the COX-2 inhibitor nimesulide alters seizure and pro- and anti-inflammatory cytokine levels in hippocampus and cerebral cortex of mice subjected to pentylenetetrazol (PTZ)-induced seizures. **Methods:** Adult male Swiss mice received vehicle (0.1% carboxymethylcellulose plus 5% Tween 80, p.o.) or nimesulide (0.2, 2 or 20 mg/kg, p.o.), daily for 14 successive days. On the 15th day mice were challenged with PTZ (50 mg/kg, i.p.). After PTZ administration animals were monitored for 20 minutes for the appearance of myoclonic jerks and generalized tonic-clonic seizures. The number of seizure episodes, total time spent seizing and Racine scale score were recorded. After behavioral analysis animals were euthanized and temporal cerebral cortex and hippocampus were dissected and homogenized according to manufacturer's protocol for posterior analysis of interleukins (IL-1β, TNF-α, INF-□, IL-6 and IL-10) by ELISA. **Results**: Subchronic administration of nimesulide significantly increased the latency to PTZ-induced generalized tonic-clonic seizures [$H(3)=8.3$; $p<0.05$]. However, nimesulide did not alter the latency to PTZ-induced myoclonic jerks, number of seizure episodes, total time spent seizing and Racine scale. Furthermore, the increase in IL-1β [$F(1,11)=32.16$, $p<0.01$], IL-6 [$F(1,11)=31.96$, $p<0.01$], INF-γ [$F(1,11)=32.25$; $p<0.01$] and TNF-α [$F(1,11)=31.75$, $p<0.01$] levels in cerebral cortex and hippocampus induced by PTZ was not prevented by nimesulide. PTZ administration increased IL-10 [$F(1,11)=61.09$, $p<0.01$] levels in cerebral cortex and hippocampus and nimesulide significantly potentiated such an increase in both cerebral structures. **Discussion:** Considering that simultaneously nimesulide decreased seizures and increased IL-10 levels in the cerebral cortex and hippocampus, and that IL-10 has been known to decrease seizures, it is possible that IL-10 increase may underlie the currently described anticonvulsant effect of nimesulide. Source of research support and acknowledgements: CAPES, CNPq, FAPERGS, PRPGP/UFSM, PIBIC/UFSM. The protocols followed the official Government Ethics Guidelines and were approved by the University Ethics Committee (N°024/2014). **References: 1.** Cazareth et al. J Neuroinflammation, 11:132, 2014; 2. Chikuma et al. J. Mol. Neurosci., 39:175, 2009; 3. Nakao et al. Mol. Cell. Biochem., 238:11, 2002; 4. Abdallah D. M. Brain Res 1351:246, 2010; 5. Kaushik et al. Exp Neurol., 257:157, 2014; 6. Gomez et al. J Neurochem, 6:770, 2014.

04.033 Tumor-associated macrophages are modulated toward a M1 phenotype by paclitaxel through a TLR-4 dependent mechanism. Wanderley CWS[1], Colon DF[2], Luiz JPM[2], Oliveira FFB[1], Viacava PR[2], Cunha TM[3], Cunha FQ[3], Lima-Júnior RCP[1] [1]UFC – Farmacologia e Fisiologia, [2]FMRP-USP – Biochemistry and Immunology, [3]FMRP-USP – Farmacologia

Anticancer mechanisms of classic cytotoxic agents commonly target cell proliferation properties. Currently, the concept that many chemotherapy drugs may elicit tumor-specific immune responses is emerging. Previous reports suggest that Paclitaxel (PCX), a drug used to treat breast cancer patients, shows a LPS-like agonistic effect on the Toll-like Receptor-4 (TLR-4). However, how this mechanism influences PCX-dependent activation of immune cell response in the tumor microenvironment is unknown. This study aimed to investigate the effects of paclitaxel on M1/M2 macrophage polarization in *in vitro* and *in vivo* (*in situ* tumor) conditions. Bone marrow-derived macrophages (BMDM) were obtained from C57BL/6 (WT) or TLR-$4^{-/-}$ mice and were incubated with PCX (10, 30, 100 µM), LPS (100 ng/mL, a M1 stimulus), IL-4 (10ng/mL, a M2 stimulus) or IL-4 plus PCX or LPS. Following a 48-h incubation period, M1 and M2 markers (TNF, IL-12, iNOS and CCL22, IGF-1, CD206/MR, respectively) were measured in cell culture supernatant. Breast cancer (4T1 cells)-bearing Balb/C mice were treated with saline or PCX (10 mg/kg, 3x 6/6h) and the frequency of $F4/80^{+}$/$CD206/MR^{+}$ cells was measured by flow cytometry. PCX increased M1 markers (TNF, IL-12, iNOS) in macrophages obtained from WT animals, but not in TLR-4 deficient cells. In addition, PCX blocked IL-4-dependent production of CCL22, IGF-1, CD206/MR, and also shifted M2-polarized macrophages towards M1 phenotype. Furthermore, tumor-associated $F4/80^{+}$/$CD206^{+}$ macrophages population was reduced when compared with saline group (CEPA: 56/2016). Therefore, this study suggests that paclitaxel shifts tumor-associated M2-like macrophages to M1 phenotype in a TLR-4 dependent-manner. **Financial support**: CNPq, Funcap.

04.034 Progression of Systemic Metabolic Alterations Induced by Colonic Inflammation in DSS-model Silveira ALM[1], Oliveira MC[2], Menezes DM[2], Rodrigues DF[2], Lana JP[2], Rachid MA[3], Ferreira AVM[2], Teixeira MM[1] [1]UFMG – Biochemistry and Immunology, [2]UFMG – Nutrition, [3]UFMG – General Pathology

Introduction: Inflammation and metabolic changes are associated and related to the development of secondary chronic disease, including atherosclerosis. The aim of the present study was to describe the progression of such link in animal model of DSS-induced colitis. **Methods:** For acute inflammation evaluation BALB/c female mice received dextran sulfate sodium (DSS) in the drinking water for a maximum of six days. Then animals were euthanized after two, four or six days of DSS; or received the chemical for six days and were euthanized 14 days after DSS removal - remission phase. For chronic colonic inflammation colitis was based of five DSS-cycles administration; each cycle was comprised of six days of the chemical followed by 14 days DSS-free period. **Results:** In the acute phase, the main systemic metabolic alteration occurred after six days of DSS administration, followed by inflammatory response in mesenteric adipose tissue. Furthermore, in acute colitis the DSS-group showed reduction in serum levels of glucose associated with an increase in cholesterol and triglycerides compared with the control group. The serum glucose reduction may be in part of the reduction of glucose-6-phosphatase expression, assayed by qPCR in liver tissue. The colitis group showed reduction in adiponectin and resistin, however an increase in leptin levels were demonstrated compared with the control group. DSS-mice showed an increase in myeloperoxidase activity, eosinophil peroxidase, and TNF-alfa, IL-6 and IL-10 cytokine levels compared with the control group in the mesenteric adipose tissue. All of these alterations induced glucose intolerance in the colitis-mice after 14 days of DSS compared with the control group. In the chronic colitis model, metabolic alteration still remained after 14 days of DSS-free interval with slightly differences from acute colitis. Glucose, cholesterol and triglycerides showed increased levels in DSS-mice compared with control. In contrast to acute colitis, there was decrease in leptin and increase in resistin levels. **Conclusion:** Taken together, persistent colonic inflammation induces the development of a metabolic syndrome-like with systemic metabolic alterations and the development of glucose intolerance. Those alterations may contribute to the increased risk of coronary artery disease in IBD patients and needs to be considered in the clinical practice. **Financial support:** Capes, CNPq, Fapemig. Local animal ethics committee protocol number: 296 / 2012.

04.035 The acute exposure to the ambient pollutant 1,2-Napththoquinone regulates human and mice eosinophil chemotaxis. Feitosa KF[1], Santos KT[1], Favaro RR[2], Santana FPR[3], Prado CM[3], Sato ASP[4], Ferreira HHA[4], Zorn TMT[2], Muscará MN[1], Costa SKP[1] [1]ICB-USP – Farmacologia, [2]ICB-USP – Biologia Celular e do Desenvolvimento, [3]Unifesp-Diadema – Biociências, [4]São Leopoldo Mandic – Inflamação

Introduction: We have previously shown that the early contact with the ambient pollutant 1,2-naphthoquinone (1.2-NQ) enhanced the innate immune responses at adulthood via direct/indirect activation of endotoxin-toll-like receptor (TLR4) pathway [1]. This study was undertaken to further determine the involved cellular mechanisms in the lung tissue of adult male wild type (WT) and TLR4 knockout (KO) mice prior exposed to 1,2-NQ during neonatal period. We also extrapolate the data on the effects of 1,2-NQ on polymorphonuclear (PMN) chemotaxis in murine to healthy human PMN *in vitro*. **Methods**: Male C57BL/6 WT and TLR4 KO neonate mice were exposed to the pollutant 1,2-NQ (100 nM) or its vehicle, accordingly [1], and were divided in 4 groups: control (vehicle), pollutant (1.2-NQ), allergic (OVA) and 1.2-NQ+OVA. Lung samples for each mouse/group were processed in order to permit the analysis of airway remodeling (H&E), mucus production and vascular cell adhesion molecule-1 (VCAM-1) expression. In parallel, healthy human eosinophil or neutrophil chemotaxis in response to 1,2-NQ (10 - 100 nM; 37ºC) incubation (20 min) was evaluated using a microchemotaxis chamber, in response to eotaxin (100ng/mL) or fMLP ($5x101^{-8}$M) [2]. **Results**: The early exposure to 1,2-NQ significantly increased the ratio muscle wall area/basal membrane perimeter (WAm/Pbm: 4.2 ± 0.6$\mu m^2/\mu m$) in the lung of allergic WT mice compared to allergic mice exposed to vehicle only (WAm/Pbm: 2.7 ± 0.2* $\mu m^2/\mu m$) or as compared to TLR4 KO mice exposed to both 1,2-NQ and OVA (WAm/Pbm: 2.7 ± 0.6*$\mu m^2/\mu m$). The early contact with 1,2-NQ in WT increased acid mucus hypersecretion (2.5 ± 1.5%) compared to related vehicle-treated mice (0.1 ± 0.3%) or TLR4 KO mice exposed to 1,2-NQ and OVA (0.5 ± 0.3%) without affecting total neutral mucus production. Immunoreaction for VCAM-1 in the lung of WT mice was higher in apical region of epithelial cells of bronchioles compared to related TLR4 KO group. Incubation (20 min) of healthy human blood eosinophils or neutrophils with 1,2-NQ (10 – 100 nM) did not significantly affect eotaxin- or fMLP-induced chemotaxis, respectively. However, 1,2-NQ by itself (100 nM) significantly increased eosinophil chemotaxis. **Conclusion**: Neonatal exposure to 1,2-NQ stimulates allergic airway remodeling in murine via a TLR4-dependent mediated pathway. Similarly to the chemotactic effects of 1,2-NQ in murine airways, the expsoure of human PMN with 1,2-NQ led to increased eosinophil chemotaxis. We suggest that the early contact with 1,2-NQ, a contaminant of particulate matter, might be a potential inductor of pulmonary allergic inflammation. Acknowledgments: CAPES, CNPq, FAPESP. Ethic committee: Numbers 1200 CEPSH and 48/2016 CEUA **References:** 1. Inflammation Research. 2011 v. 60. p. S174-P276. 2. BMC Pulm Med. 2008 Aug 12;8:13. 3. J Immunol Methods 1991;145:105–10.

04.036 Tumor necrosis factor-alpha reduces platelet aggregation independently of IKK, but dependently of PKCδ or PKCε activation. Bonfitto PHL, Bueno PI, Naime ACA, Antunes E, Marcondes S Unicamp – Farmacologia

Introduction: Platelets have been described as important cells in inflammation, however, the effects of cytokines on platelet reactivity are rarely studied. Tumor necrosis factor-alpha (TNF-α) is an essential mediator in the pathogenesis of many inflammatory and cardiovascular disorders and part of its effects occurs through NF-kB. Platelets possess many elements involved in the TNF-α signaling. Our group observed that this cytokine reduced platelet aggregation through TNFR activation, which was accompanied by Ca^{2+} mobilization reduction and inhibition of c-Src and fibrinogen receptor activation, but the mechanisms involved in these effects were not elucidated yet. Therefore, the objective of the present work was to determine the signaling pathway involved in the inhibitory effect of TNF-α on platelet aggregation. **Methods:** Blood from abdominal aorta of male Wistar rats (250-320g) was collected in ACD-C (9:1 v/v). Platelet-rich plasma (PRP) was obtained by centrifugation of whole blood at 200 g for 15 min. The platelets were washed using citrated buffer (pH 6.0) and the number was adjusted to $1.2x10^8$ plat/ml. Platelet aggregation was measured in a two channel aggregometer (Chronolog Lumi-Aggregometer). Aggregation assays were carried out incubating platelets with TNF-α (0,01-3000 pg/ml) for 30min or 24h before ADP addition. In some experiments the platelets were pre-incubated with the inhibitors of IKK (IKK16, 0,01 - 3μM), PKC δ (rottlerin, 5μM), or PKCε (SC3095, 1 and 10μM) before TNF addition (100 pg/ml). **Results:** Incubation of platelets with TNF-α for 30min reduced ADP (5μM)-induced aggregation in a dose-dependent manner. TNF-α (100 pg/ml, 30 min) inhibited about 60% of the ADP-induced platelet aggregation. Concentrations lower than 1 pg/ml of TNF-α, did not modify platelet aggregation compared to control group, even when platelets were incubated with TNF-α for longer period of time (until 24h). Pre-incubation of platelets with the IKK inhibitor IKK16 (3μM, 20 min) significantly inhibited ADP (5 μM)-induced aggregation (60 ± 10%, n=7). The inhibitory effect of TNF-α (100 pg/ml) on platelet aggregation was increased by 20% in presence of IKK16. Inhibition of PKCδ and PKCε did not affect platelet aggregation. On the other hand, pre-incubation of platelets with rottlerin or SC3095 before TNF-α addition, reduced the inhibitory effect of this cytokine on aggregation (10 ± 3% of aggregation in absence of PKC inhibitors and about 25 ± 2% of aggregation in presence of either PKCδ or PKCε inhibitors). **Conclusion:** Nuclear factor kappa-B (NFκ-B) positively modulates ADP-induced platelet aggregation. However, this transcription factor does not take part in TNF-α inhibitory effect on platelet aggregation. Additionally, PKCδ and PKCε activation partially contributes to the platelet aggregation inhibition by TNF-α. **Financial support:** CNPQ **Research approval process number:** 3709-1

04.037 Role of regulatory T-cells in Irinotecan-induced intestinal mucositis. Fernandes C[1], Wanderley CWS[1], Muniz HA[1], Silva CMS[1], Teixeira MA[1], Souza NRP[1], Cândido AGF[1], Ribeiro RA[1], Almeida PRC[2], Lima-Júnior RCP[1] [1]UFC – Farmacologia e Fisiologia, [2]UFC – Patologia e Medicina Legal

Introduction: Intestinal Mucositis(IM) is a common side-effect associated with irinotecan(IRI) therapy for colorrectal cancer. IM can cause delayed chemotherapy cycles and treatment interruption. The involvement of inflammatory mediators, such as TNF-α, IL1-□, IL-18 and KC, has been demonstrated by our group. However, the role of adaptive immune system cells is yet to be shown in IM. Aim:To evaluate the role of Tregs in IRI-induced IM. **Methods**: C57BL/6(20-25g) were injected with saline(n=10) or IRI(75 mg/kg, i.p.) once a day/4 days. The mice were euthanized at day 1(D1,n=4), 3(D3,n=6), 5(D5,n=4) or 7(D7,n=4) following the first dose of IRI. For Tregs depletion, the mice were pretreated with a low single dose of cyclophosphamide(100 mg/kg, CYP), 2h before the first IRI administration and euthanized at D7. Intestinal lamina propria lymphocytes were harvested by enzymatic digestion and purified by Percoll. The frequency of Th subsets was identified by flow cytometry. Blood leukocyte count ($x10^3$/□l) was obtained and ileum samples were collected for histopathological analysis and myeloperoxidase assay (MPO, neutrophil/mg tissue). ANOVA/Bonferroni's, Mann Whitney or Pearson test were used for statistical analysis.*P*<0.05 was accepted. **Results**: IRI-injected mice presented diarrhea on D5 and D7 when compared to saline [SAL: 0(0-0); D5: 0(0-2), P<0.05; D7: 1(0-3), P<0,0001]. Intestinal neutrophils increased along mucositis development, which peaked on D5(21171± 15289, P<0.01) and decreased on D7(6163±4304;P<0.01) when compared to control(1873±571.7). Intestinal Tregs showed a 3x and 7x increase, respectively on D5(8.7±6.2 vs SAL:2.8±2.5;P<0.05) and D7(18.7±6.2% vs SAL: 2.8±2.5;P<0.0001). Similar increase was seen to intestinal Th17 cells (SAL: 9.19±3.3%; 2x on D5: 22.4± 4.9%;P<0.01; 3x on D7: 28.2±8.3; P<0.01). The number of neutrophils decreased on D7 when % Tregs peaked. Indeed, a negative correlation between these cells in IRI group was observed(r= -0.71;P=0.02). The %Th17 cells did not have correlation with neutrophils in these mice (r= -0.35; P=0.31). To evaluate the hypothesis that Tregs are important to control of inflammation in IM, mice were depleted of Tregs with CYP. %Tregs was reduced to baseline levels in CYP+IRI group when compared to IRI group(CYP+IRI: 1.3±0.7% vs IRI: 18.7±6.2%, P<0.0001). Tregs depletion reduced animal survival with IM(CYP+IRI[0% survival] vs IRI[50% suvival],p=0.01). Tregs depletion induced a marked weight loss (P<0.0001), increased diarrhea[2(0-3),P<0.01], intestinal damage (villus/crypt ratio-P<0.01) and intestinal accumulation of neutrophils (22254±9203;P<0.0001), when compared to IRI group. The % of other $TCD4^+$ subsets ($CD3^+CD4^+CD25^-FOXP3^-$) increased in CYP+IRI group vs IRI group (P<0.0001) with a strong negative correlation with %Tregs (r= -0.98;P<0.0001).**Conclusions**: Tregs are important to control of inflammation in IRI-induced IM, probably by negatively modulate neutrophils and Th subsets. Financial Support: CNPq/CAPES/FUNCAP. Animal Research Ethical Committee:75/2013.

04.038 The tyrosine kinase inhibitor dasatinib inhibits airway inflammation, mucus exacerbation and peribronchial fibrosis in a mouse model of asthma non- responsive to glucocorticoids. Serra MF[1], Cotias AC[1], Pimentel AS[1], Arantes ACS[1], Silva PMR[1], Rocco P[2], Martins MA[1] [1]Fiocruz – Fisiologia e Farmacodinâmica, [2]UFRJ

Introduction: Glucocorticoids are the mainstay of therapy in asthma, but glucocorticoid resistance is a challenging clinical problem in a significant proportion of severe asthma patients. Dasatinib is a dual Src/Bcr-Abl tyrosine kinase inhibitor developed for the treatment of chronic myeloid leukemia, and able to inhibit proliferation, activation, and function of T lymphocyte, macrophage and dendritic cells. In this study, we attempted to assess the impact of dasatinib interventional treatment upon pathological changes triggered by repeated allergen provocation in a long-term mouse model of asthma non-responsive to glucocorticoids. **Methods:** Mice of strain A/J were subcutaneously sensitized on days 0 and 14 by a suspension of Al(OH)3 and ovalbumin (OVA), and challenged for nine consecutive weeks, once a week, starting on the 3rd week post-sensitization. Administrations of dexamethasone (1 mg/kg) or Dasatinib (10 mg/kg) were given orally for seven consecutive days in the last week of the once-a- week series of OVA provocations. Airway hyper-reactivity (AHR), cytokine generation, extracellular matrix deposition, mucus exacerbation, and oxidative stress were evaluated 24 h after the last challenge (CEUA license # L-030/15). **Results:** Dasatinib, but not dexamethasone, inhibited allergen-induced eosinophil and neutrophil accumulation in the BAL fluid, under conditions where the increased levels of mononuclear cells were refractory to both treatments. Increased lung tissue levels of IL-4, TNF-α, eotaxin-1 and -2, KC, TARC and MIP-1α, noted in asthmatic mice, as well as the peribronchial fibrosis were also significantly inhibited only by dasatinib treatment. Furthermore, while both dasatinib and dexamethasone inhibited mucus exacerbation equally, none of them modified allergen-induced AHR or changes in lung tissue levels of catalase and TBARS. **Conclusion:** Our findings show that dasatinib interventional therapy can significantly inhibit at least part of pivotal pathological changes triggered by recurrent allergen provocation, including lung remodeling, in a murine model of asthma non-responsive to glucocorticoids. These effects were likely due to down-regulation of pro-inflammatory cytokines and chemokines and subsequent blockade of eosinophilic and neutrophilic infiltration in the lung tissue. **Financial Support**: CNPq, FAPERJ, MS/DECIT, TARKINAID, TIMER and CAPES.

04.039 The role of neutrophils in the chronification of the immune response using an antigen induced arthritis model. Uribe-Alvarez R, Amaral FA, Teixeira MM UFMG – Biochemistry and Immunology

Introduction: Neutrophils are known to contribute greatly to the acute inflammatory response but there is still need for studies regarding its role in the chronification of the immune response. **Methods**: We used a model of antigen induced arthritis (AIA) to study neutrophils from different inflammatory phases (peak and resolution) and tissues (lymph node, spleen and knee joint). We evaluated neutrophils activation state and surface protein profile using flow cytometry. **Results**: We obtained a clear difference in expression of surface proteins like MHCII and CD86 between cells from different inflammatory phases. We also blocked neutrophil recruitment with a CXCR2 receptor antagonist and saw that this altered the surface protein expression as well. **Conclusion**: Our results show that neutrophil surface molecule expression changes in the chronic immune response leading us to think that neutrophils might have a different role in this inflammatory phase. **Financial Support**: National Council for Scientific and Technological Development (CNPq) Project approved by the etics and animal research committee of the UFMG under the protocol number 86/2014.

04.040 Comparison of bone regeneration in male and female Type 1 Diabetic mice: effects of Vitamin D supplementation. Cignachi NP[1], Machado GDB[2], Ribeiro A[1], Silva RBM[2], Campos MM[1] [1]PUCRS – Odontologia, [2]PUCRS – Medicina

Introduction: Complications related to diabetes can affect many organs. We have previously demonstrated that type 1 diabetes (T1D) leads to impaired bone healing, by affecting osteoblast activity (Cignachi et al., J Cell Physiol. 230: 3019, 2015). The aim of this study was to evaluate the bone healing in an experimental model involving a femoral defect, in male and female type 1 diabetic mice. The effects of vitamin D supplementation were assessed in this model. **Methods:** Male and female C57BL/6JUnib mice (18-25 g) were used. The local animal ethics committee (15/00433) approved the experimental protocols. Type 1 diabetes was induced by 5-daily injections of streptozotocin (STZ; 50 mg/kg i.p.), dissolved in citrate buffer (50 mM; pH 4.5). Non-diabetic groups received only citrate buffer vehicle i.p., at the same time-points. The bone defects were created 7 days after the last STZ injection. After anesthesia with ketamine and xylazine (100 plus 10 mg/kg, i.p.), the left mouse femur was assessed, and a monocortical bone defect (4-mm in length and 1-mm in diameter) was created. The animals were distributed into four experimental groups, subdivided in male and female: (1) non-diabetic mice treated with vehicle; (2) non-diabetic mice treated with vitamin D3; (3) STZ-diabetic mice treated with vehicle; (4) STZ-diabetic mice treated with vitamin D3. Vitamin D3 (4 mg/kg) was administered orally, once a day, during 21 days. On day 33, the animals were euthanized, and the femurs were collected for hematoxylin-eosin (H&E) staining. A semi-quantitative analysis of the percentage of newly formed bone in comparison to the total analyzed area was carried out. An Elisa assay was performed to determine the serum interleukin-6 (IL-6) levels. **Results:** Diabetes induction in female and male mice led to a significant decrease of body weight, accompanied by hyperglycemia, in relation to the non-diabetic groups. Based on the evaluation of H&E sections, type 1 diabetic mice presented reduced areas of newly formed bone tissue (15.4 ± 1 % and 18.9 ± 5.3 %), when compared to non-diabetic mice (34.8 ± 3 % and 30 ± 2.4 %), in female and male mice, respectively. Of note, the long-term supplementation with vitamin D3 led to an increase of bone healing areas in type 1 diabetic females (25.13 ± 8.3 %), but not in diabetic male mice (18.9 ± 5.3 %). There were no significant differences regarding the serum IL-6 levels, when comparing male and female mice, irrespective of the experimental group ($P>0.05$). **Discussion:** Present data allow us to suggest that bone regeneration displays a distinct profile in non-diabetic female and male mice, whereas the induction of type 1 diabetes led to impaired bone healing in both sexes, regardless of IL-6 serum levels. Interestingly, the administration of vitamin D3 was able to increase the newly formed bone tissue only in female diabetic mice. Further experiments are in progress to extend these results, and for better evaluating the quality of newly bone in different experimental conditions. **Financial support:** PUCRS, PROBIC/FAPERGS CAPES, CNPq, FINEP.

04.041 Pyruvate kinase M2 (PKM2), an isoenzyme of the glycolytic pathway, is pivotal to the development of psoriasis. Veras F[1], Prado D[1], Melo B[1], Tartari P[1], Melo P[1], Costa L[2], Cecilio N[1], Publio G[1], Alves M[3], Lima D[4], Nakaya H[4], Sales K[3], Souza C[2], Cunha F[5], Alves-Filho JC[5] [1]FMRP-USP – Farmacologia, [2]FMRP-USP – Clínica Médica, [3]FMRP-USP – Biologia Celular e Molecular, [4]FCF-USP – Análises Clínicas e Toxicológicas, [5]CRID-FMRP-USP

Introduction: Psoriasis (Ps) is a chronic inflammatory disease that affects the skin, which is characterized by infiltration of Th17 cells and hyperproliferation of keratinocytes. Recent studies on the role of metabolic pathways in regulation of immune cell function have brought the increasing area of immunometabolism. In this context, pyruvate kinase M2 (PKM2), an enzyme that regulates the final step of glycolysis, can also translocate into the nucleolus and control pro-inflammatory gene expression. However, the role of this pathway in the development and maintenance of the inflammatory response in Ps is unclear. According to above, we study aims to evaluate the role of PKM2 in the development on experimental psoriasis. **Methods AND Results:** Data mining of GEO datasets revealed that PKM2 expression was significantly increased in psoriatic lesional skin samples, but was normal in uninvolved skin of patients, which positively correlated with the expression of pro-inflammatory (IL-17A, IL-22 and IL-23) and metabolic (HIF-1α and LDH) genes. Immunofluorescence staining confirmed the overexpressed of PKM2 and HIF-1α in epidermis of lesional skin from psoriatic patients. To investigate the role of PKM2 in the development of Ps, psoriasis-like skin inflammation was induced by topical application of imiquimod (IMQ) on the back skin of C57BL/6 mice treated or not daily with shikonin (SKN), a PKM2 inhibitor (4 $mg.kg^{-1}$, i.p). SKN strongly ameliorated the development of psoriasis-like skin inflammation and reduced the levels of Th17 cytokines (IL-17, IL-22 and IL-23) in the dorsal skin. Moreover, H&E and immunofluorescence staining of skin sections showed that SKN significantly ($p<0.05$) reduced acanthosis, leukocytes infiltration and keratin 17 (K17) and S100A9 expression compared with the control group. Finally, flow cytometry analysis showed reduced frequency of Th17 cells after treatment with SKN. **Conclusion:** Our results showed that PKM2 is overexpressed in skin lesional of psoriatic patients and it´s inhibition ameliorates experimental psoriasis. Accordingly, PKM2 playing a pivotal role to development or maintenance of experimental psoriasis, with future clinical implications.

04.042 Plasmin induces macrophage reprogramming and contributes to features of inflammation resolution. Ribeiro ALC, Sugimoto MA, Costa BRC, Vago JP, Lima KM, Carneiro FS, Ortiz MMO, Lima GLN, Carmo AAF, Rocha RM, Perez DA, Reis AC, Pinho V, Miles LA, Teixeira MM, Garcia CC, Sousa LP UFMG

Plasmin (Pla) is produced by the liver in an inactive form, plasminogen (Plg), and it plays a vital role in protecting the host from thrombotic events. In addition to acting in fibrinolysis the Plg/Pla system components play an important role in cell migration and therefore can regulate the inflammatory response. Resolution of inflammation is an active process triggered early during inflammation whose main goal is to restore tissue homeostasis. Although the participation of the plasminogen/plasmin (Plg/Pla) system on the productive phase of the inflammation is well know their involvement on resolution phase is unclear. This study investigated the implication of Plg/Pla in key events during resolution of acute inflammation and the underlying mechanisms. The injection of Plg/Pla into the pleural cavity of BALB/c mice induced a time-dependent influx of mononuclear cells that were primarily macrophages of anti-inflammatory (M2 - F4/80^{high} Gr1^{-} CD11b^{high}) and pro-resolving (Mres - F4/80^{med} CD11b^{low}) phenotypes, without changes in the number of macrophages with pro-inflammatory profile (M1 - F4/80^{low} Gr1^{+} CD11b^{med}). There was increased expression of CD206 and Arginase-1 (M2 markers) and increased levels of IL-6, IL-10 and TGF-β in the pleural cavity without effecting iNOS (M1 marker) expression. During the resolving phase of LPS-induced inflammation, when M2 and Mres macrophages predominate, we found increased plasminogen expression and plasmin activity suggesting a link between Plg/Pla system and macrophage reprogramming. Indeed, Plg or Pla given at the peak of inflammation promoted resolution by decreasing neutrophils numbers and increasing their apoptosis and efferocytosis. Plg and Pla increased efferocytosis of apoptotic neutrophils by murine macrophages and the levels of the pro-efferocytic protein Annexin A1 (AnxA1). Interestingly, the increased efferocytic capacity observed in BALB/c mice after Plg treatment was lost in AnxA1$^{-/-}$ mice. These results suggest that Plg/Pla can act on several checkpoints of the inflammation resolution, neutrophil apoptosis, macrophage reprogramming and efferocytosis, which have an impact on setting up an efficient resolution process. Financial Support: Fapemig, CNPq, CAPES and PRPq-UFMG All procedures described here had prior approval from the Animal Ethics Committee of Universidade Federal de Minas Gerais (CETEA/UFMG, Protocol number: 19/2011).

04.043 Increased reactive oxygen species formation in platelets of lipopolysaccharide-injected mice is dependent on tumor necrosis factor-alpha production. Naime ACA, Sollon C, Bueno PI, Bonfitto PHL, Lopes-Pires ME, Anhê GF, Antunes E, Marcondes S FCM-Unicamp – Farmacologia

Introduction: The role of platelets in sepsis has been increasingly studied. Sepsis severity has been correlated to the platelet activation. Sepsis condition may be induced by lipopolysaccharide (LPS) that triggering biological responses by activating specific cells including platelets. During sepsis occurs great generation of reactive oxygen species (ROS), which has been implicated in multiple organ failure. Tumor necrosis factor-alpha (TNF-α) levels is greatly increased in sepsis and in animals injected with LPS. In addition, TNF-α increases NADPH oxidase activation, that is an important ROS generation source in different cells. Therefore, we decided investigate the role of TNF-α in the increasing ROS production in platelets of LPS-injected mice. **Methods:** The present study was approved by the Committee for Ethics in Animal Research (State University of Campinas – UNICAMP, protocol number 3290-1). ROS generation in non-activated or ADP-activated platelets was determinate by flow cytometry using DCFH-DA. For *ex vivo* experiments, C57BL/6J mice were injected (i.p.) with saline, LPS (1 mg/kg) or TNF-α (10ng/kg) and after 48h the animals were anesthetized with isoflurane for blood collection (cardiac puncture) and ROS determination. In some experiments, the animals were pre-treated with the antibody anti-TNF-α infliximab (10 mg/kg, s.c.), or with the non-specific antagonist of TNF-α receptor R-7050 (6 mg/kg., i.p.) or with the NADPH oxidase inhibitor apocynin (85 mg/kg by gavage) before LPS or TNF-α injection. For *in vitro* experiments, non-activated or ADP-activated platelets were incubated with saline or TNF-α for 5, 30 or 60 min to measure ROS formation. In some experiments, platelets were pre-incubated with with infliximab (1µg/ml), R-7050 (1nM) or apocynin (100µM) before incubation with saline or TNF-α. **Results:** Incubation of ADP-activated platelets with TNF-α (3000 pg/mL, 30 min) increased 4.0-fold ROS production compared to the platelets in absence of this cytokine. Infliximab abolished ROS generation in platelets incubated with TNF-α. Additionally, the antagonist of TNF-α receptor RI/R2 R-7050 reduced ROS in platelets incubated with TNF-α by 40%. Inhibition of NADPH oxidase by apocynin reduces in 50% the increased ROS production by TNF-α in platelets. Treatment of mice with LPS or TNF-α caused a significant increase of ROS in platelets compared to platelets of mice injected with saline (increased by 3.7- and 4.2-fold, respectively). ROS generation induced by LPS was abolished by the pre-treatment of mice with infliximab, while R-7050 reduced by 50%. When the animals were pre-treated with apocynin, LPS-induced ROS production in platelets was inhibited by 44%. Similarly, the increased ROS levels in platelets of TNF-α-injected mice was abolished by infliximab and significantly reduced by both R-7050 and apocynin. **Conclusion:** TNF-α is an important cytokine involved in the increased ROS production in platelets of mice injected with LPS **Supported by:** CAPES

04.044 A new animal model of radiation proctitis induced by high-dose rate brachytherapy: possible involvement of IL-6 and IL-8 Leite CHB[1], Lopes CDH[2], Leite CAVG[1], Terceiro DA[1], Freitas JA[1], Wong DVT[3], Almeida PRC[3], Cunha FQ[4], Lima-Júnior RCP[2]
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Introduction: Radiotherapy, including teleradiotherapy and brachytherapy, is one modality of anticancer treatment, which is based on ionizing radiation to induce tumor cell death. Such therapeutic approach is employed in approximately 65% of all cancers. In the brachytherapy modality, the radiation source is positioned in contact with the tumor mass. Radiation proctitis is a common toxicity (5-20%) related to brachytherapy of prostate, gynecological and rectum cancers with a known negative impact on patient's quality of life. The underlying mechanisms are unknown due to the lack of an adequate animal model that mimics clinical radiation proctitis. Then, the present study aimed to develop an animal model of brachytherapy-induced proctitis in mice. **Methods**: C57BL/6 male mice (20-25 g, n=6/group) were subjected to high dose rate radiotherapy [radiation source: iridium-192 (Ir-192)] through a cylindrical propylene tube with a 4 mm outer diameter and the tip inserted 2 cm far from anal sphincter into the rectum. The radiation doses used were 7.5 or 9.5 Grays (Gy) for three consecutive days. The sham group received only the applicator with no radiation source. Body weight variations, diarrhea scores, survival rate, perianal lesion scores and rectal damage were performed at 24 and 48 hours and on seventh and thirtieth days after animal irradiation. Samples of rectum were collected for histopathologic analysis and dosage of cytokines (IL-1, IL-6 and IL-8). Unpaired Student t-test, Mann Whitney or log rank tests were used for statistical analysis. $P<0.05$ was accepted. **Results**: The animals that received radiation at 9.5 Gy dose showed a 66% mortality and significant weight loss on day 30 versus sham group ($P<0.05$). There was no difference between irradiated and sham groups ($P> 0.05$) at 24h, 48h or on day 7 over these parameters. Irradiated group (9.5 Gy) also showed increased perianal lesions, rectum damage assessed through colonoscopy, histopathology scores and cytokine levels (IL-8 and IL-6) in comparison with the sham group, which was more prominent on the seventh day ($P<0.05$). IL-1 levels remained at basal levels ($P>0.05$). Animals that were irradiated with a 7.5 Gy dose showed no signs of damage compared to sham group ($P>0.05$). **Conclusion**: We developed an animal model of radiation proctitis induced by high-dose rate brachytherapy, which mimics clinical disease. **Financial support**: Funcap, Capes, CNPq. This study was approved by local ethics committee (protocol number Nº 50/13).

04.045 Biocompatibility evaluation of polypyrrole using zebrafish as a model organism. Costa KM[1], Soares JC[1], Valente CA[2], Cruz FF[3], Basso NRS[2], Bogo MR[1] [1]PUCRS – Biologia Celular e Molecular, [2]PUCRS – Química, [3]PUCRS – Farmacologia

Introduction: Zebrafish (*Danio rerio*) is an animal model increasingly used in biomedical research including human toxicology (Raldúa et al., *Reprod Toxicol* 33, 188, 2012) and one of the most promising *in vivo* model systems for toxicity screening (Bohnsack et al., ***Methods*** *Mol Biol* 926, 261, 2012). Recently, zebrafish have been used to evaluate the toxicity of several nanomaterials (Wang et al., *Biomed Environ Sci* 28, 341, 2015) because is a facile model for the rapid evaluation of the potential toxicity and biodistribution of nanomaterials (Fako et al., *Adv Drug Deliver Rev* 61, 478, 2009). After the discovery that electrical signals can regulate cell attachment, proliferation and differentiation (Rivers et al., *Adv Funct Mater* 12, 33, 2002), researches sought to incorporate conducting polymers into biomaterials to take advantage of electrical stimuli, and the polypyrrole (Ppy) has been widely studied in biomedical applications (Tian et al., *Prog Polym Sci* 37, 237, 2012). The present study aimed at analyzing the biocompatibility of Ppy, after exposition of different concentration of nanomaterial in zebrafish larvae. **Methods**: The Animal Ethics Committee (CEUA 15/00479) approved all the protocols. The zebrafish embryos were exposed to the dispersion Ppy in the first 4 h post-fertilization (hpf) to 144 hpf. *Survival Curve:* Larvae mortality was evaluated in the groups (Control, Buffer, 25, 100, 250 and 500 µg/mL Ppy) in 24, 48, 72, 96, 120 and 144 hpf after particulate Ppy exposure (30 larvae per group). *Embryo toxicity test:* The embryonic spontaneous movement (1 min) were monitored with the aid of a microscope at the time point of 24 hpf (10 embryo per group) and the frequency of the heart bates of embryo/larvae (1 min) were monitored with the aid of a microscope at the time point of 48 hpf (10 embryo/larvae per group). *Statistical Analysis:* We used Kaplan-Meier method for the survival curve and One-way Analysis of Variance (ANOVA) followed by Tukey's test in the spontaneous movement and cardiac rate. Data were expressed as mean±standard error and $P<0.05$ was considered significant. **Results**: To evaluate the possible toxicity of the suspensions of Ppy in different concentrations to zebrafish embryos, the mortality was analyzed and a significant reduction in the larvae survival in the doses of 500 µg/mL it was noted after 144 h exposure, featuring 70% mortality. The spontaneous movement analysis shows a trend of increasing movements of animals in the groups treated with higher concentrations of the nanomaterial. However, so far has not found any statistical difference in relation to the treated groups when analyzing the average heart beats. **Conclusion**: Additional experiments are in progress to evaluate the potential inflammatory or toxic of Ppy, and will be useful to establish quality in the development of biomaterials in the field of regenerative medicine. FINANCIAL SUPORT: CAPES and CNPq.

04.046 Assessment of neutrophil chemotaxis in patients with severe sepsis or septic shock admitted an Intensive Care Unit. Resende C[1], Rezende B[1], Borges I[2], Carvalho E[1], Santos A[2], Nobre V[1], Pinho V[1], Teixeira MM[1] [1]ICB-UFMG, [2]UFMG

Introduction: Sepsis is a systemic inflammatory syndrome caused by infection and is the major cause of death in intensive care units in the world. The investigation of inflammatory mediators, recruitment of neutrophils and cell signaling pathways has been an important research line. The failure of neutrophils migration for infection sites may be associated to systemic spread of the pathogen, resulting in the release of inflammatory mediators. Previous studies in septic patients have shown an association between reduced neutrophil migration capability and chemotactic G protein-coupled receptor due increased protein expression kinases that induce phosphorylation GPCR. Herein, we assessed in this work, the clinical profile of patients with severe sepsis and septic shock in a Intensive Care Unit at UFMG and the neutrophil migration in the human sepsis. **Objectives:** To evaluate the clinical profile of patients with severe sepsis and septic shock admitted to the Intensive Care Unit of the Hospital das Clinicas-UFMG and to evaluate the neutrophil migration capability in cells humans by chemotaxis expression of CXCR1, CXCR2, GRK2, GRK5 and pAKT in human neutrophil of patients with severe sepsis and septic shock. **Methods:** A Cohort study was conducted between October 2014 to December 2015 in the ICU of the Hospital das Clinics-UFMG. All patients fulfilled criteria for severe sepsis or septic shock. **Results:** Forty-three patients were included for clinical analysis. Neutrophil chemotaxis and flow cytometry assays were performed in six patients. Patients severity was evaluated according to APACHE II, SAPS3 and SOFA and range was respectively, 19.5, 62.8 and 9.35. The average age was 58 (18-97) and 55.8% (24) female and 44.2% (19) male. Thirty-nine patients (90.7%) were diagnosed with septic shock. Our preliminary data showed that 83% of the patients survived at 28 days. Relevantly, there was a reduction of 40% of neutrophil chemotaxis in patients with severe sepsis or septic shock compared to health individuals. The CXCR1, GRK2 and GRK5 were increased in these patients. **Conclusion:** GRK2 and GRK5 pathway may be are related with the neutrophil chemotaxis reduction. Better understanding of this pathway will be therapeutically useful in the clinical management of patients with sepsis.

04.047 Anti-inflammatory synergistic effect of diclofenac associated with terpinolene on subchronic inflammation in rats. Macedo EMA[1], Piauilino CA[1], Santo WC[1], Sousa Neto BP[1], Reis Filho AC[1], Sousa DP, Oliveira FA[1], Almeida FRC[2] [1]UFPI, [2]UFPI – Bioquímica e Farmacologia

Introduction: The inflammatory process is a response to various stimuli, which leads to edema formation and hypersensitivity as a result of cell and liquid infiltration in injured tissues. Pharmacological treatment of inflammation is usually done with anti-inflammatory non-steroidal agents (NSAIDs) with good efficacy, but with significant adverse reactions. There is need to search alternatives such as NSAIDs and natural products association with synergistic anti-inflammatory action and reduced side effects. So, the aim of this study was to investigate the synergistic anti-inflammatory effect of sodium diclofenac (DCF) and terpinolene (TPL, monoterpene from several essential oils) association. **Methodology:** Wistar rats (170-230 g/n=6-9)(CEEA/UFPI, Nº. 82/2014) received 50 µL of complete Freund's adjuvant (CFA) into the hind paw. After 24 hours all rats were treated orally with TPL (3.125; 6.25; 12.5 and 25 mg/Kg); DCF (1.25; 2.5 and 5 mg/Kg); the association of DCF-1.25 plus TPL-3.125 mg/Kg or vehicle (Saline), followed by the evaluation of paw volume by plethysmometer, during 0, 1, 2, 3, 4, 5 and 6 h, as well as every 24 h for 10 days. For differential leukocyte count after the subchronic treatment (D10), the rats were euthanized and all of the right hind paws were removed, processed for histopathological evaluation and visualized with an optical microscope (increase of 1,000x). For each animal were randomly selected 10 fields, followed by the sum of the cells. The leukocyte populations assessed included neutrophils, macrophages and lymphocytes. The statistical analysis used two-way ANOVA, followed by Bonferroni´s test, $p<0.05$. **Results:** In the acute phase, DCF, TPL and the association of DCF/TPL not significantly reduced the paw edema when compared to the control. In subchronic phase, DCF at the doses 1.25 and 2.5 mg/kg also did not reduce paw edema, however DCF in the dose 5 mg/kg reduces edema, the first (D1) 0.37 ± 0.04 mL to tenth day (D10) 0.28 ± 0.03 mL in the control; only the highest dose of TPL (25 mg/kg) reduced the paw edema in the last four days of evaluation, where this reduction was 0.40 ± 0.01 ml (D7); 0.36 ± 0.01 mL (D8); 0.34 ± 0.01 mL (D9) and 0.32 ± 0.02 ml (D10), all were statistically significant when compared to the control group (0.49 ± 0.02 mL); already DCF/TPL association reduced the paw edema of the fifth (D5) to tenth day (D10), and these five days the statistical difference was the same in the control. The smallest reduction of edema was in D10: 0.34 ± 0.02 ml. For differential leukocyte count, the group treated with BPD (25 mg/kg) significantly reduced only macrophages (8.60 ± 1.21 cells / 10 random fields), compared to the control group (30.60 ± 4.31 cells). DCF decreased macrophages (16.40 ± 2.50 cells) and lymphocytes (280.00 ± 14.94 cells). Since the DCF / TPL association reduces the infiltration of the three types of cells: 46.48% neutrophils, 81.05% macrophages and 48.50% lymphocytes. **Conclusion:** The association DCF / TPL showed synergistic anti-inflammatory effect on chronic inflammation, with reduced infiltration of immune cells into the inflamed tissue. **Financial support**: UFPI/CAPES

04.048 Atypical chemokine receptor ACKR2 contributes to the development of lung fibrosis in silicotic mice. Dias DF[1], Correa AMC[1], Pereira JG[1], Arantes ACS[1], Cordeiro RSB[1], Graham G[2], Martins MA[1], Silva PMR[1] [1]Fiocruz – Inflammation, [2]University of Glasgow – Infection, Immunity and Inflammation

Introduction: Silicosis is part of a group of pulmonary pathologies consequence of a long-term exposure to inhaled dust of silica, characterized by a slow progressive fibrosis and impairment of lung function. In spite of the therapeutic arsenal currently available, there is no specific treatment for the disease. Chemokines are the principal regulators of leukocyte migration and act through binding conventional signaling receptors. In addition there also exists a small subfamily of atypical chemokine receptors (ACKRs)2, which binds with high affinity, essentially to all inflammatory CC-chemokines but does not mount classical signaling responses following ligand binding. **Aim**: The purpose of the present study was to investigate the importance of the receptor-type decoy ACKR2, previously known as D6, in the context of experimental silicosis in mice. **Methods**: Silica particles (10 mg/50 µL) or saline (control) were instilled by intranasal route into C57BL/6 ($ACKR2^{+/+}$) and knockout mice ($ACKR2^{-/-}$), and the analyses performed at 7 (initial phase) or 28 (late phase) days post-stimulation. The parameters included included: lung expression of ACKR2 in the lungs by PCR; lung function and airways hyper-reactivity to methacholine by invasive plethysmography and morphology/morphometry by classical hematoxylin & eosin technique. The reactivity of macrophages (peritoneal) *in vitro* was also performed by ELISA. All procedures were approved by the Ethics Committee on Animal Use (CEUA) of Fiocruz in the LW57/14 license. **Results**: Silica-challenged mice exhibited a time-dependent leukocyte infiltration in the lung parenchyma and granuloma formation during the course of the disease. The mRNA expression of ACKR2 was shown to be lower in the silicotic lungs than those from controls, at 7 and 28 days. Silica exposure also caused an increase in the basal levels of lung resistance and elastance as well as airways hyper-reactivity to aerosolized methacholine. $ACKR2^{-/-}$ mice showed a less intense inflammatory response, including granuloma formation, and displayed significantly reduced airways hyper-reactivity as compared to control group. In another set of experiments, we demonstrated that macrophages recovered from the peritoneal cavity of $ACKR2^{-/-}$ mice exhibited reduction of TNF alpha generation when stimulated with LPS and silica *in vitro*. **Conclusion**: Our data indicate that lung function alterations and granulomatous fibrosis were attenuated in silicotic $ACKR2^{-/-}$ mice, a phenomenon which paralleled with lower macrophage responsiveness *in vitro*. These findings are in contrast to those published in the literature regarding dermal dysfuntions and suggest that the atypical receptor ACKR2 seems to act differently depending on the site or organ affected. More experiments are needed in order to clarify the role of ACKR2 in lung fibrotic diseases such as silicosis. **Financial support**: FIOCRUZ, CNPq, FAPERJ and European Community (UE FP7- 2007-2013 - n°HEALTH-F4-2011-281608).

04.049 Alpha-1-Acid glycoprotein inhibits human neutrophil response by a sialic acid dependent mechanism. Lorenzini CB[1], Cardoso F[1], Colón D[2], Cunha FQ[2], Spiller F[1] [1]UFSC – Immunobiology, [2]FMRP-USP – Inflammation and Pain

Introduction: Neutrophil recruitment has a central role in host response and in resolution of inflammation, but if uncontrolled can lead to severe tissue damage. In response to a chemotactic gradient, neutrophils extravasate and chemotax toward the site of inflammation. Upon encountering chemotactic stimuli, neutrophils are activated leading to a calcium influx and F-actin polymerization. The acute phase protein alpha-1-acid glycoprotein (AGP) has been shown to inhibit some steps of the neutrophil migration process such as rolling and adhesion *in vivo, and* chemotaxis *in vitro.* The mechanism by which AGP inhibits the neutrophil chemotaxis is not completely understood. In this regard, AGP activity is related to its carbohydrate portion, and the lost of its sialic acid residues implicates in the lacking of their capacity to modulate the leukocyte response. Therefore, we hypothesized that sialic acid residues of AGP are essential for it's inhibitory effect on human neutrophils. In this study we investigate if AGP impairs chemotaxis, actin polymerization and calcium influx by a sialic acid dependent mechanism. **Methods**: Human neutrophil chemotaxis was stimulated by fMLP in a modified Boyden chamber assay. Actin polymerization was observed with a Phalloidin immunofluorescence assay, and the calcium influx evaluated using Fluo-3AM protocol. To investigate the influence of sialic acid residues, AGP treated with neuraminidase (which cleaves sialic acid in α-2,3 - α-2,6- or α-2,8) was used in the assays mentioned above. **Results**: AGP (250µg/ml) inhibits human neutrophil chemotaxis *in vitro*, actin polymerization and calcium influx induced by the chemotactic factor fMLP. Furthermore, AGP-treated neuraminidase significantly reversed the protein suppressive effect on neutrophils. Conclusion: Our results suggest that AGP inhibits the neutrophil migration process through the inhibition of chemotaxis, actin polymerization and calcium influx by a sialic acid dependent mechanism. **Financial support**: CAPES, CNPq, FAPESP and PPGF-UFSC

04.050 Effects of augmented O-GlcNAcylation on activation and differentiation of macrophages. Zanotto CZ[1], Olivon VC[2], Pereira CA[1], Mestriner FLAC[1], Alves-Filho JC[1], Carneiro FS[1], Tostes RC[1] [1]FMRP-USP – Farmacologia, [2]Uniderp

Introduction: Macrophages (MØs) play an important role in regulating the immune system, especially in the initial phase response. They can be activated via receptors for components of microorganisms and cytokines. MØs are classified as classically-(M1) or alternatively-(M2) activated, based on their exposure to different fate-determining mediators. The post-translational modification of proteins by *O*-GlcNAcylation (*O*-GlcNAc) is highly dynamic and modulates cell-signaling processes. Chronic conditions that increase the levels of *O*-GlcNAc-modified proteins are associated with vascular disorders. However, acute increases in *O*-GlcNAc levels reduce the release of pro-inflammatory mediators and regulate inflammatory processes by decreasing e.g. NF-□B activation. **Aim:** This study tested the hypothesis that increased *O*-GlcNAc levels favor polarization of MØs to the anti-inflammatory/M2 phenotype. **Methods:** Macrophages obtained from bone marrow of male BALB/c mice were incubated with vehicle, glucosamine, the *O*-GlcNAcase inhibitors PugNAc (100 µM) and Thiamet-G (TMG, 1µM), lipopolysaccharide (LPS, 1 mg/ml) + interferon-g (IFN-□, 200ng/ml) for M1 or interleukin-4 (IL-4, 50ng/ml) for M2 polarization. Expression of polarization markers (F4/80 and CD206) was assessed by flow cytometry. **Results:** Cellular viability was not affected by all compounds tested, as measured by MTT reduction assay. Incubation of MØs with TMG, glucosamine and PugNAc produced a time-dependent increase in *O*-GlcNAc levels, determined by western blot. Incubation of undifferentiated MØs with TMG significantly increased IL-1□ release (control= no detected, TMG 38.9 ± 2.5* pg/mL) after 24h, as well as with LPS (1 mg/mL, 40.7 ± 1.4* pg/mL). In M1-differentiated MØs, stimulation for 24h with TMG further increased IL-1□ (30.01 ± 4.4*) and IL-6 (7.26 ± 0.2*) mRNA ($2^{-\Delta\Delta CT}$) expression. TMG increased levels expression of IL-1β (control= 36.28 ± 2.3, TMG= 82.03 ± 6.9* pg/mL) and TNF-□ (control= 271.8 ± 9.2, TMG= 325.1 ± 0.6* pg/mL) in supernatant of M1-differentiated MØs. Levels of $[Ca^{2+}]_i$ were measured in undifferentiated MØs following stimulation ATP (1mM), in the presence of incubation with vehicle or 1mM TMG by 24h. TMG inhibited the increases in cytosolic Ca^{2+} induced by the purinergic agonist ATP in macrophages (control= 3209 ± 185.9, TMG= 1120 ± 130.6* AUC); **Results** are presented as mean ± SEM for *n* = 4-6 in each experimental group. One-way ANOVA followed by Bonferroni's post-test. *, *P*< 0.05 vs. control. **Conclusion:** These preliminary results suggest that increases in *O*-GlcNAcylation in 24h contribute to a pro-inflammatory phenotype in macrophages. Our studies suggest that the *O*-GlcNAc pathway as a potential therapeutic target in diseases associated with inflammatory responses. **Financial Support:** CNPq, CAPES, CRID and FAPESP. Approval of Animal Research Ethical Committee: protocol number 019/2013.

04.051 Evaluation of immunomodulatory effect of essential oil obtained from *Siparuna guianensis* Aublet towards lymphocytes obtained from mice bearing experimental autoimmune encephalomyelitis *in vitro*. Alves JV[1], Silva SKS[1], Silva CA[1], Silva AM[2], Rovarotto CF[3], Silva GA[3], Silva IR[1], Santos LMB, Farias AS[3], Parise MR[1] [1]UFG, [2]IFC, [3]Unicamp

Introduction Multiple Sclerosis (MS) is a chronic, inflammatory and demyelinating autoimmune disease that affects Central Nervous System thus leading to axonal damage and visual, motor and/or sensory disorders[1]. Although its ethiology remains unknown, it seems to be mediated by $TCD4^+$ cells and a subset of $TCD4^+$ cells with regulatory properties (T_{REG}) is thought to be involved in the control of its progression[2]. Experimental Autoimmune Encephalomyelitis (EAE) is an experimental model of MS largely used for its comprehension[3]. There is not a cure for MS, however, there are medicines able to suppress the immune response in an inespecific way, thus increasing the risk of long-term side effects[4]. Siparuna guianensis (SG) essential oil, due to its anti-inflammatory properties[5], may be a potential candidate for MS treatment. It was performed a preliminary evaluation of SG essential oil in order to assure its efficacy at cellular level for future in vivo studies. The cytotoxic in vitro effects of SG essential oil towards lymphocytes obtained from lymph nodes of mice at the onset of EAE was evaluated for 24 and 48 hours. **Methods** EAE was induced in C57BL/6 mice by immunization with a MOG_{35-55} emulsion in Freund's complete adjuvant and by the administration of Bordetella pertussis toxin after 48h. Mice were observed daily in order to analyze the clinical evolution of EAE and when mice had lost their tail tonus (10th day post-immunization), mice were sacrificed and had their axial lymph nodes removed. The obtained cells were cultivated in microplates and then incubated with concentrations of SG essential oil ranging from 200 to 1.563µg/mL for the cytotoxicity evaluation through the reduction test of 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT)[6]. The flow cytometry technique was employed to characterize T_{REGs}, by using the anti-CD3, CD4, CD25 and FoxP3 antibodies. **Results** SG essential oil presented a concentration-dependent cytotoxicity, being 6.25µg/mL the lowest concentration capable of keeping 100% of lymphocytes viability, both after 24 and 48h exposition, and significantly increased ($p<0.05$) the relative number of $T_{REGs,}$ which seems to be implicated in a better clinical course of EAE and MS[2]. **Conclusion** Overall data obtained showed an immunoregulatory property of SG essential oil but further studies, mainly in vivo, are necessary to evaluate this effect and overall security of SG essential oil. References: [1]STADELMANN, C. **Curr Opin Neurol**. 24:224, 2011. [2]O'CONNOR, RA. **J. Neuroimmunol.** 193:1, 2008. [3]GOLD, R. **Brain**. 129:1953, 2006. [4]KEVIN, J. P. **Mol. Pharm**. 11:828, 2014. [5]LEITE, G.O. **Fitoterapia**. 82: 208, 2011. [6] MOSMANN, T. **J Immunol Methods:** 65: 55, 1983. **Financial support**: FAPESP and CNPq This research was approved by the Ethics Committee on Animal Use of UNICAMP (Protocol n. 3601-1)

04.052 Calcitonin-Gene Related Peptide (CGRP) is a potent mediator of edema in the rat cheek. Almeida MPA[1], Queiroz BFG[1], Bakhle YS[2], Francischi JN[1] [1]UFMG – Farmacologia, [2]Imperial College London – Leukocyte Biology

Introduction: Carrageenan, injected into the mucosal tissue of rat cheeks, induced a long-lasting edema persisting for more than 24h (Frade et al., 2016). As there is extensive sensory innervation of orofacial tissue, we aimed to verify the reactivity of this tissue, in terms of edema formation, to CGRP, one of the sensory neuropeptides present in these fibers (Escott and Brain, 1993). **Methods**: Male Wistar rats weighing 150-200 g, anesthetized with isoflurane (Isoforine), were injected with CGRP into the right side of the mouth at time zero. A range of doses of CGRP (in 0.1 ml) was used and increase in the thickness of the cheek (in mm), relative to the contralateral cheek (injected with saline and shown as Δthickness) was measured with a digital caliper (Mitutoyo, Japan) before and after 5, 15, 30 min and 1, 2, 3, 4, 6 and 24 h of the injection. Control animals were injected with 0.1 ml saline, under similar conditions. Data are shown as mean$\pm$ sem (n=5) at each time and significant differences between means were assessed by one-way ANOVA, when P <0.05. **Results**: CGRP induced a dose-dependent edema in the cheek, using much lower doses (8-33 pmol) than those used for SP (0.7–37 nmol), with a longer duration of action (at 6 h=$CGRP_{16pmol}$=1.78$\pm$ 0.07mm; SP_{7nmol}=0.42$\pm$0.07). In addition, 10 nmoles of the compound $CGRP_{8-37,}$ an antagonist of CGRP, given locally 10 min before the agonist prevented the cheek edema, as compared with control animals ($CGRP_{8-37}$+CGRP=0.36+0.13; SAL+CGRP=1.25$\pm$0.06mm). **Conclusions**: The sensory neuropeptide CGRP is able to induce edema in the orofacial tissue by activation of specific receptors. Due to its potency and efficacy to induce cheek edema, CGRP should also be considered an important mediator of inflammation in this tissue. **References**: 1. Frade et al (2016) - Tissue-selective inflammation in the oral cavity of the rat. Inflammopharmacology (accepted, DOI: 10.1007/s10787-016-0269-0). 2. Escott JK, Brain SD (1993) – Effect of a calcitonin gene-related peptide antagonist (CGRP8-37) on skin vasodilatation and oedema induced by stimulation of the rat saphenous nerve. Br J Pharmacol 110:772-776 **Financial support**: CNPq and FAPEMIG Experiments were approved by the UFMG Animal Use Ethics Committee with the protocol number 368/2014.

04.053 Unpredicted lethality of substance P administered intraorally in ketamine-xylazine anesthetized rats. Queiroz BFG[1], Almeida MPA[1], Frade TIC[1], Bakhle YS[2], Francischi JN[1]
[1]UFMG – Farmacologia, [2]Imperial College London – Leukocyte Biology

Introduction: We have found that carrageenan injected into the cheek tissue of rats anesthetized with a ketamine+xylazine mixture, induced a dose-dependent and long-lasting edema (Frade et al., 2016). Given the extensive sensory innervation of orofacial tissue, we aimed to assess the contribution of the endogenous sensory neuropeptide, substance P, to this edema. **Methods**: Holtzman and Wistar male rats, weighing 150-200g, were anesthetized either with a ketamine-xylazine combination (K+XYL=60+15 mg/kg) or with inhaled isoflurane (Isoforine) to permit an intraoral injection of substance P (SP: 1-50 µg/animal), histamine (H: 100µg) or serotonin (5-HT: 5µg) in the right side of the mouth. In other groups of animals, the corresponding antagonists (SP: SR140333; H; pyrilamine; 5-HT: pizotifen at 2-4 mg/kg) were injected subcutaneously, 30 min before the agonist. We also administered the highest dose of SP (50µg) to the hind paws, using both rat strains. Increase in cheek or paw thickness (in mm) in relation to the contralateral side injected with saline, was taken as a measure of edema. Data are presented as mean±sem (n=5) at each time; significant differences were determined by one-way ANOVA when P <0.05. **Results**: Unexpectedly, 64% (7/11) of the Holtzman rats and 80 % (4/5) of the Wistar rats anesthetized with K+XYL, injected intraorally with 50µg SP died within 15min of injection, whereas there were no deaths (0/5), using 1µg SP. Death was accompanied by signs of excessive salivation. Pretreatment with SR140333 reduced mortality (2/5; 40%) after 50µg SP. However, mortality after 50µg SP injected intraorally was completely prevented when rats were anesthetized with isoflurane. Histamine and 5-HT injected intraorally in rats of either strain anesthetized with the K+XYL mixture caused no deaths and induced the predicted edema, which was reversed by their corresponding antagonists. Moreover, SP (50□g) injected in the hind paw of non-anesthetized rats also caused no deaths and induced edema. Conclusion: The unexpected and high lethality of intraoral SP is restricted to the concomitant use of the K+XYL anesthetic, as deaths did not occur with other edemagenic mediators or after isoflurane anesthesia. The K+XYL anesthetic mixture, widely used in rodents, should not be used when experiments involve the administration of SP to the orofacial tissue of rats.

Reference: Frade TIC, Dos Reis DC, Cassali, GD, Bakhle YS, Francischi JN (2016) - Tissue-selective inflammation in the oral cavity of the rat. Inflammopharmacology (accepted, DOI: 10.1007/s10787-016-0269-0). **Financial support**: CNPq and FAPEMIG This study was approved by the local Animal Ethics Committee with the Protocol numbers 97/2013 and 368/2014.

04.054 Aloe Vera extract delays mortality but does not attenuate kidney injury after cecal puncture and ligation in mice. Yasojima EY[1], Dórea MA[1], Yamaki VN[1], Teixeira RKC[1], Feijó DH[1], Gouveia EHH[2], Feitosa Junior DJS[1], Valente AL[1], Carvalho LTF[3], Franco RC[2], Leite GMO[1], Neto ESM[1] [1]UEPA, [2]Cesupa, [3]UFPA

Introduction: Intensive Care Units (ICU) admissions due to sepsis represent 2 to 11% of all occurrence rates. Although the great therapeutic intervention available, it is the major cause of death in ICU and the leading cause of acute kidney injury. The choice of antibiotics and the appropriate time to initialize vital support measures (such as mechanical ventilation and vasoactive drugs) is a challenge because of the difficult clinical management caused by hemodynamic and electrolyte changes resulting from the sepsis. The aim of this study is to evaluate the role of Aloe Vera extract on survival rates and kidney histopathology of mice submitted to cecal ligation and puncture. **Methods**: The Ethics Committee in the Use of Animals of the State University of Para (UEPA) approved the research. Twenty-six male mice were used, weighing between 35 – 45 g. The animals were randomly distributed into four groups: 1) Sham – surgical simulation (N=5); 2) Control - performed cecal ligation and puncture (N=7); 3) Aloe – surgical simulation treated with Aloe Vera extract (N=7); and 4) Sepsis + Aloe – performed cecal ligation and puncture and treated with Aloe Vera extract (N=7). The animals were observed until death. After the death was confirmed, the kidneys were collected for the histopathological analysis. Kruskal-Wallis test was used to compare the histopathological Results: Survival analysis was assessed by Kaplan-Meier test and compared using log-rank test. It was adopted a significance level of 5%. **Results**: The survival time ranged from 19 hours for the control group to 24 hours for the animals of Sepsis + Aloe Vera group, corresponding to a survival time 1.26 times better ($p<0.01$). There was no difference between Sham and Aloe groups, neither between Control and Sepsis + Aloe groups according to the four parameters analyzed ($p>0.05$). **Conclusion**: In conclusion, the Aloe vera extract delays mortality, but does not attenuate kidney injury induced by cecal ligation and puncture in mice. **Financial support** and acknowledgements: **Financial support** from LCE/UEPA. The Ethics Committee in the Use of Animals approved the research, protocol 03/15. **References**: ANGUS, D.C. Crit Care Med. v.29, p.109, 2001 BELE, R. Infection. v.37, p. 222, 2009 BOTELHO, N. M. Acta Cir Bras. v.29, p.5281, 2014 CHOI, S. Semin.Integr. Med. v.1, p.53, 2003 GURIB-FAKIM, A. Mol Aspects Med. Feb; v.27, p.1, 2006 HAMMAN, J.H. Molecules. v.13, p. 1599, 2008 HOLDER, A.L. Crit Care. v.17, p. 309, 2013 HSU, D.Z. Shock. v.22, p.582, 2004 KIM, K. Phytomedicine. v.16, p.856, 2009. LOPES, L.N. Acta Cir Bras. v.30, p. 568, 2015 MOLAZEM, Z. Glob J Health Sci. v.7, p. 203, 2014 RAHMANI, N. Eur Rev Med Pharmacol Sci. 2014;18(7)p.1078, 2014 RANIERI, V.M. N Engl J Med. v.366, p.2055, 2012. SOGAYAR, A.M. Pharmacoeconomics. v.26, p.425, 2008. STOLLER, J. J Crit Care. v.31, p.58, 2016 VÁSQUEZ, B. J Ethnopharmacol.v.55, p.69, 1996 WALLEY, K.R. Infect Immun. v.64, p.4733, 1996 WANG, H. J Nutr. v.136, p.360, 2006 WESTPHAL G.A. Rev Bras TerIntensiva. v.23, p. 13, 2011 YUN, N. Food ChemToxicol. v. 47, p.1341, 2009 ZHOU, F. Crit Care Med. v.42, p.270, 2014.

04.055 Influence of hydrogen sulfide (H2S) on expression and function of adhesion molecule on human neutrophil and eosinophil. Salamí YAM[1], Sato ASP[1], Feitosa KB[2], Costa SKP[2], Ferreira HHA[1] [1]Faculdade São Leopoldo Mandic – Inflammation Research, [2]ICB-USP – Pharmacology

Introduction: Hydrogen sulfide (H_2S) has showed beneficial effects in allergic airways diseases by reducing *in vivo* eosinophil and neutrophil migration to the lungs of sensitized mice after allergic challenge. However, the influence of H_2S on the expression and function of adhesion molecules involved in leukocyte migration is unknown. Thus, the aim of this study was to investigate whether H_2S modulates the functionality of adhesion molecules expressed on human eosinophil (EO) and neutrophil (NE) by chemotaxis and adhesion *in vitro* studies. The expression of the adhesion molecules VLA-4 (CD49d/CD29), Mac-1 (CD11b/CD18) and LFA-1 (CD11a/CD18) on these cells was also evaluated. **Methods:** All experiments were approved by the Animal Ethics Comitee/SLMandic (license n. 917.360). EO and NE obtained from healthy volunteer were pre-incubated with slow-releasing (GYY4137-3.0mM) or fast H_2S releasing (sodium hydrosulfide; NaHS -1000µM). Control cells were pre-incubated only with RPMI 1640. Chemotaxis of EO and NE to eotaxin (10^{-8}M) and fMLP ($5x10^{8}$M), respectively, was carried out in a ChemoTx-5 chamber. Adhesive properties of these cells were performed using fibronectin-coated plates. EO and NE were quantified by eosinophil peroxidase (EPO) and myeloperoxidase (MPO) activities, respectively. Adhesion molecule expression was analysed by flow cytometry. **Results:** The presence of NaHS or GYY4137 was able to attenuate the EO and NE chemotaxis [NaHS (EO: 0.23□0.03; NE: 0.1□0.02) and GYY4137 (EO: 0,24□0.04; NE: 0.20□0.09)] compared to respective controls (EO: 0.4□0.02; NE: 0.8□0.1). Equally donors decreased about 40% the EO adhesion to fibronectin. Neutrophil adhesion was reduced in 77% and 80% by NaHS or GYY4137, respectively. Adhesion molecules on cells of control group (non-treated cells) showed that 99,5% of eosinophils expressed CD11a, CD11b and CD49b. The frequency of neutrophil $CD11a^{+}$, $CD11b^{+}$ and $CD49d^{+}$ was 99.8, 99.5 and 94.6, respectively. Although the expression of CD11a was higher on eosinophils ($15.2x10^{3}$□1.7 versus $5.7x10^{3}$□0.9 on neutrophil), the CD11b is the main adhesion molecule on neutrophil ($11.0x10^{3}$□0,5 versus $5.6x10^{3}$□0.5 on eosinophil). CD49d increased expression was observed among eosinophil and neutrophil ($4.9 x10^{3}$□0.5 versus $3.6 x10^{3}$□ 0.3; $p<0.05$). **Conclusion:** The results suggest that H_2S reduced the eosinophil and neutrophil chemotaxis and adhesion but not adhesion molecules expression. Whether the impaired migration is due to modulation of CD106, CD54, CD62P and CD63E (integrin ligant) on endothelial cells is under investigation. Support: Fapesp; CNPq

04.056 Evaluation of anti-inflammatory activity of LASSBio-1827. Nascimento TS, Freitas RHCN, Fraga CAM, Fernandes PD, Cordeiro NM UFRJ – Farmacologia e Química Medicinal

Introduction: Inflammation is a response to infections or tissue damage leading to restoration of the structure and tissue function[1]. LASSBio-1827 was synthesized from LASSBio-1524, an inhibitor of IKK-β enzyme[2]. Aim: To evaluate the anti-inflammatory effect of LASSBio-1827. **Methods**: Mice (n=6-8) were pre-treated with LASSBio-1827 in doses of 10, 30, 100 µmol/kg in *in vivo* experiments. The licking paw model induced by formalin is characterized by two phases: nociceptive (1^{st}) and inflammatory (2^{nd}). Mice received an injection of formalin (20 µl) into the hind paw 1 hour after treatment with LASSBio-1827 and the time that the animal licked the paw was recorded. The model of subcutaneous air pouch (SAP) was done to assess cell migration. Mice received injection of sterile air (10 ml) into the intrascapular area. After three days reinforcement was performed to maintain the pouch. On 6^{th} day mice received an injection of carrageenan suspension (1%) 1hour after treatment with LASSBio-1827. After 24 hours euthanasia was performed and the exudate was collected for total leukocyte count and determination of plasma proteins. To determinate the production of reactive oxygen species (ROS), cells were treated with LASBio-1827 (1,10,30 µM) 1 hour before stimulation with PMA. Finally, the probe DCFH-DA was added. The production of ROS was evaluated by the emission fluorescence captured in the FL1 flow cytometer channel. Control: dexamethasone (0.3 µmol/kg) intraperitoneally. Statistical analysis was done by ANOVA/Bonferroni test and results are expressed as mean ± SD and ($p < 0.05$) *. **Results**: Licking paw model induced by formlain: 1^{st} phase: 10 µmol/kg =27 ± 11seg; 30 µmol/kg=24 ± 9sec; 100 µmol/kg=32,5 ± 11,7 sec; vehicle = 21,9 ± 6sec. 2nd phase: : 10 µmol/kg =179 ± 87sec; 30 µmol/kg=142,2 ± 36sec; 100 µmol/kg=96,4 ± 25,6sec*;vehicle=198 ± 6.0sec.In SAP: 10 µmol/kg = 17.7 ± 17 celsx10^6 / ml*, 30 µmol/kg = 18 ± 9.8 celsx10^6 / ml*,100 µmol/kg = 9.17 ± 11.4 cels 10^6 / ml* compared to the carrageenan group = 63.16 ± 20.4 celsx10^6 / ml; vehicle group = 5.22 ± 3.9 celsx10^6 / ml; control group = 26.5 ± 7.7 celsx10^6 / ml. Extravasation of plasma proteins: 10 µmol/kg= 101.7 ± 95.5 µg/ml*, 30 µmol/kg=177.4 ± 73.9 µg/ml, 100 µmol/kg = 105.1 ± 79.2 µg/ml* compared to carrageenan group = 256.9 ± 102.2 µg/ml; vehicle group = 20.19 ± 30.4 µg/ml. ROS production: 1 µM = 1,02 x10^5 ± 9,65x10^2, 10 µM= 3,05 x10^5 ± 8,1x10^3*,30 µM= 1,06 x10^5 ± 4,1x10^3* compared to the group stimulated with PMA = 1,44 x10^5 ± 23,4x10^3. Group unstimulated = 3,8 x10^5 ± 5,4x10^2. **Conclusions**: The results indicate that LASSBio-1827 has anti-inflammatory effect because it reduced the paw licking time, cell migration, extravasation of plasma proteins and ROS. LAWRENCE, T.; WILLOUGHBY, D.A.; GILROY, D.W. Anti-inflammatory lipid mediators and insights into the resolution of inflammation. Nature Immunology. 2: 787-795. 2002. AVILA, C.M.; LOPES, A.B.; GONÇALVES,A.S.; SILVA, L.L.; ROMEIRO, N.C.; MIRANDA, A.L.P.; SANT'ANNA, C.M.R.; BARREIRO, E.J.; FRAGA, C.A.M. Structure-based design and biological profile of (E)-N-(4-Nitrobenzylidene)-2-naphthohydrazide, a novel small molecule inhibitor of IκB kinase-β. European Journal of Medicinal Chemistry. 46: 1245-1253. 2011. **Acknowledgements**: Alan Minho for technical assistance, Institute Vital Brazil for donation of mice. **Financial support**: CAPES, CNPq and FAPERJ. Ethics committee of the CAUAP/UFRJ protocol number:DFBCICB015-04/16.

04.057 Padronization of an experimental model of induced pulmonary emphysema by inhaled cigarette smoke. Silva TS, Souza ACS UFSJ

Introduction: The aim of this work was to standardize a model of pulmonary emphysema, in mice, induced by inhaled cigarette smoke. The proposed model of inhalation of tobacco smoke can aid, in the future, the prospection of novel drugs for emphysema treatment and the better understanding of the disease's patophysiology. MATERIALS AND **Methods**: Male C57BL/6 adult mice (n=8, 28-32 g) were exposed to the smoke amount of three cigarretes, three times a day, during 7 (acute) or 30 (subchronic) days. The inhalation apparatus was developed by members of the Pharmacology Laboratory – Federal University of São João Del Rei (UFSJ), Campus CCO, based on the model described by KOZMA *et al.,* 2014. Controls were exposed to the same conditions of fresh air. One day after the last exposure, five animals of each group were euthanized, and the bronchoalveolar lavages (BAL) were collected using 3 X 0.5mL saline phosphate buffer (PBS) solution. Approximately 2.0mL BAL was recovered for each animal and total leucocyte numbers were counted in Neubauer chamber with Turk's staining solution. The last three animals of each group were also euthanized one day after last cigarette exposure and the lungs collected for Haematoxillyn and Eosin (HE) histological analysis. **Results**: Several changes in the histological lung architecture were observed among animals of both exposed groups, such as: pulmonary lesions characterized by interalveolar septa inflammation, located along the bronchic tree; enlargement of septa with edema, diffuse inflammatory infiltrate, punctual hyperemia (7 days of exposure) and hemorrhagic areas (30 days of exposure). Moreover, the differences in number of leucocytes were statistically significant for both treated groups, when compared to the respective controls. **Conclusions**: This model of pulmonary emphysema induction has to be adjusted to become more trustable and reproducible. It was also noted that different exposure times lead to distinct pulmonary lesions in mice, as observed in HE microscopy. However, the observed findings suggest that the apparatus induced lung inflammation and injury, characteristics of the pulmonary emphysema. **References**: VALENÇA, S.S. J Bras Pneumol. v. 34, p. 787, 2008.; KOZMA, R.H. J Bras Pneumol, v.40, p.46, 2014; TAMIMI, A. Respiratory Medicine. v. 106, p. 319, 2012. MORSE, D. Annual Review of Physiology, v. 76, p. 493, 2014. The tests with animals were performed in accordance with the Brazilian National Council for the Control of Animal Experimentation and approved by the Ethics Committee for Animal Research of the Federal University of São João del-Rei (protocol number: 67/2015).

04.058 Endothelin plays a proinflammatory role in primary cultures of rat lung microvascular endothelial cells activated by LPS. Silva MM[1], Balbino AM[1], Gil NL[1], Azevedo GA[1], Fernandes L[1], Landgraf MAV[1,2,3], Landgraf RG[1] [1]Unifesp-Diadema – Ciências Farmacêuticas, [2]USP – Farmacologia, [3]Unip

Introduction: The levels of endothelins in the lung tissue are particularly high compared to other tissues. Within the lung endothelins are synthesized by the epithelial cells of the airway, endothelial cells and inflammatory cells. Vascular endothelium is closely related with the circulatory control, and has an important participation in cellular and molecular events which occurred during immune system reactions and tissue injuries. **Methods:** Male Wistar rats were euthanized with overdose of ketamine and xylazine, and lung tissue samples were harvested under sterile conditions. Endothelial cells were characterized by immunofluorescence using ULEX and von Willebrand factor, which is a traditional marker of endothelial cells, and also by flow cytometry using antibodies CD54 (ICAM-1), CD105 (endoglin), CD106 (V-CAM) and CD45 (hematopoietic cells). Endothelial cells were stimulated or not with LPS (1µg/mL), and treated by endothelin A receptor antagonist (ETA - BQ123, 10ng/mL) or endothelin B receptor antagonist (ETB - BQ788, 10ng/mL). After 6 hours of incubation PGE_2 production (EIA), COX-2 expression (western blot) and the production of cytokines (multiplex) were evaluated. **Results:** Endothelial cells were positive for all markers, except for CD45. Endothelial cells treated by ETA or ETB failed to elicit any alteration on inflammatory parameters evaluated. LPS-treated endothelial cells produced high levels of PGE_2, IL1-β, IL-2, TNF-α and GMCSF, and pretreatment with ETB prevented this effect on PGE_2 and IL1-β levels. LPS induced strong the COX-2 expression (207%), and pretreatment with ETA or ETB reduced it. **Conclusion:** Our preliminary results suggest that endothelin plays an important pro-inflammatory role in in primary culture of rat lung microvascular endothelial cells, stimulated by LPS. **Financial support:** FAPESP (2010/01404-0, 2012/51104-8) and CNPq. Animal Research Ethical Committee: CEUA Nº 3096230615

04.059 Intrauterine undernourishment downregulates COX-2 and TLR-4 expression in the second generation of rats. Arakaki CP[1], Silva MM[1], Balbino AM[1], Gil NL[1], Azevedo GA[1], Ramos APA[1], Landgraf RG[1], Landgraf MAV[1,2,3] [1]Unifesp-Diadema – Ciências Farmacêuticas, [2]USP – Farmacologia, [3]Unip

Introduction: Adverse environmental factors in the prenatal period cause changes in the normal pattern of growth and development of the fetus. This can permanently affect the structure and physiology of several tissues and organs. This adaptive response includes changes in hemodynamics, metabolism, and production of hormones and their receptors, which predisposes the individual to cardiovascular, metabolic and endocrine diseases. In the present study we investigated the possible mechanisms involved in reducing the pulmonary inflammatory response induced by LPS in second generation (F2-UR) of F1 intrauterine undernourished rats, at 12 weeks of age. **Methods**: Male wistar rats at 12 weeks of age were divided into 2 groups: nourished (ad libitum diet) and F2 (ad libitum diet) obtained of F1 offspring from mothers receiving 50% of the nourished diet of counterparts. Control group was given saline intranasally (i.n., *200mL). Experimental groups were given LPS (i.n.,* 750 µg/animal*).* 6h after instillation, the bronchoalveolar lavage fluid (BALF) was collected to evaluate cellular infiltration in lung. Lungs were harvested for measurement of the cyclooxygenase 2 (COX-2), toll like receptor 4 (TLR-4) and glucocorticoid receptor (GR-R) expression by western blot. **Results:** Nourished (NR) and F2 undernourished (F2-UR) groups showed a similar increase in both total cell and neutrophils in bronchoalveolar lavage fluid after LPS instillation. Western blot assay showed that expression of COX-2 in LPS stimulated groups is decreased in F2-UR group when compared to the NR group. After LPS instillation, different from F2-UR offspring, NR offspring presented reduction in GR expression. Besides, F2-UR rats also exhibited a reduced expression in TLR-4 following LPS administration, while in the NR group no difference was observed. **Conclusion:** Our preliminary results suggest that intrauterine undernourishment downregulates COX-2 and TLR-4 expression in the F2 generation. **Financial support:** FAPESP (2010/01404-0, 2012/51104-8, 2015/22674-9) and CNPq Animal Research Ethical Committee: CEUA 1408220915.

04.060 Adrenalectomy reverses the decreased lung inflammation presented by low birth weight rats. Azevedo GA[1], Gil NL[1], Silva MM[1], Fernandes L[1], Landgraf MAV[2,3,4], Landgraf RG[1]
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Introduction: Undernourished during pregnancy may affect fetal development and impair the maturation of the immune system, exerting prolonged negative effect on the immune response. Understanding the role of corticosterone in the inflammatory response may be of great relevance in development of new therapies for the treatment of inflammatory and autoimmune diseases. In previous studies, we found that low birth weight rats, at 12 weeks of age, presented reduced acute inflammatory response associated with reduction in ICAM-1 expression and increase in circulating corticosterone levels. The present study used this model to investigate the influence of corticosterone on acute pulmonary inflammatory response. **Methods:** At 12 week of age, low birth weight male rats (LBW, obtained from dams that were fed 50% of the nourished diet of counterparts) and intrauterine nourished male rats (NR) were adrenalectomized (ADX) and replaced with corticosterone (i.p.,3 mg/kg/day) or saline for seven consecutive days. Then, the acute lung injury induced by LPS intratracheal instillation (750 mg/200mL) was evaluated: the bronchoalveolar lavage fluid was collected and cellular infiltration into lung tissue was analyzed. Lungs were harvested for measurement of cytokine production (multiplex) and glucocorticoid receptor expression and COX-2 (western blot). **Results:** Total and differential cell counts from the bronchoalveolar lavage, and histopathological analysis showed that that reduction in corticosterone levels could be restored pulmonary neutrophilic infiltration and COX-2 expression, in lung tissue. After LPS challenge, no difference was found in IL-1β and IL-6 levels, and TNF-α was increased in LBW rats. . LBW presented high basal levels of corticosterone and were not modified by LPS challenge, different from that observed with NR. LPS challenge was also not able to induce alterations in ACTH and insulin levels, in LBW rats; in turn, reduction in corticosterone levels restored ACTH and insulin levels, in LBW rats. The reduced basal expression of glucocorticoid receptors observed in LBW rats is also restored after adrenalectomy and corticosterone replacement. **Conclusion:** Our data suggest the influence of high basal concentrations of corticosterone on reduced inflammatory response developed by LBW rats, since adrenalectomy restored acute lung inflammatory response in this group. **Financial support:** FAPESP (2010/01404-0, 2012/51104-8), CNPq and CAPES. **Animal Research Ethical Committee:** CEUA Nº 1566150416

04.061 Effect of photobiomodulation on cell viability and inflammatory mediators on myoblasts submitted to *B. jararacussu* snake venom (BJSSUV) David AC, Silva LMG, Zamuner SF, Cogo JC, Zamuner SR Uninove

Background: Local myonecrose is a common consequence in envenoming caused by *Bothrops* snakes genus. Antivenom therapy and other first-aid treatments do not reverse local myonecrose. Thus, there is an urgent need to find therapies that can complement antivenoms in the neutralization of local tissue damage. **Objective:** This study analyses the effect of low level laser (LLL) on proliferation and expression of IL-1□, IL-6, IL-8, in myoblast submitted to injury by BjssuV. **Methods:** C2C12 myoblast were divided into tubes according to each experimental group and received the venom (12.5 mg/mL) or culture medium alone (control) and were centrifuged. Cells were irradiated at the bottom of the tube for 13 s with a laser at 635 and 830nm, 4.6 J/cm2 dose and power of 100 mW. Viability was analyzed by Alamar Blue for 3 up to 72 h and IL-1□, IL-6, IL-8 expression were evaluated by immunohistochemistry after venom incubation for 15, 30 and 60 min. **Results:** BjssV caused an decrease in the viability of C2C12 cells from 3 up to 72 h at 30 and 60 min of venom incubation. LLL caused an increase in cell viability by both laser studied at 30 and 60 min of venom incubation. The venom caused an increase in IL-1□ and IL-6 in C2C12 cells 60 min after venom incubation, the laser treatment was able to decrease the expression of IL-1□ but not IL-6. **Conclusion:** BjssuV is toxic to muscle cells. The LLL therapy protects against this effect.

04.062 Effect of a high-calorie / westernized diet on pharmacological effectiveness of nimesulide in Wistar rats. Araújo RB[1], Menezes TM[1], Franco ES[1], Nascimento E[2], Maia MBS[1], Araújo MGP[1], Santana LD[1], Pereira CFC[1], Cunha CCS[1], Lima LCAS[1] [1]UFPE – Farmacologia de Produtos Bioativos, [2]UFPE – Nutrição

Introduction: Obesity is a chronic and multifactorial disease triggered by rampant consumption of high-calorie foods. Epidemiological studies suggest a close relationship between obesity and chronic noncommunicable diseases (NCDs) such as hypertension, dyslipidemia and diabetes, often coexisting with inflammatory processes. Objective: To evaluate the pharmacological efficacy of nimesulide in rats fed a high-calorie / westernized diet (WD - characterized by having a higher saturated fat percentage (35%) when compared to standard diet for laboratory animals (SD - 11%). **Methods**: 20 males Wistar rats (200 - 250g) were used, divided into four groups (n = 5 animals / group) according to the nutritional manipulation: i) SD-c – fed a SD; ii) WD-c group - fed a WD; iii) SD-n – fed a SD and treated with Nimesulide (5 mg/kg, i.p.) and iv) WD-n – fed a WD and treated with nimesulide. To evaluate the anti-inflammatory effect of nimesulide, SD-n and WD-n groups received a subplantar injection of carrageenan (CAR - 0.1 ml; 1% w / v) in the left rear paw 45 minutes after administration nimesulide. The control groups (SD-c and WD-c) were subjected to the same procedure as above, but received vehicle (carboxymethylcellulose - 5 ml / kg, ip) instead of nimesulide. The inflammatory edema volume was measured by plethysmometer, at times 0 and 30, 60, 120, 180 and 240 minutes after injection of CAR. The experimental protocol was approved by the Ethics Committee on Animal Experiments of Biological Sciences Center, Federal University of Pernambuco (Protocol 23076.003.845/2015-63). **Results**: The intervals 180 and 240 minutes when compared to their respective controls, anti-oedematogenic effect of nimesulide in animals fed the WD was significantly ($p < 0.05$) lower (WD-n - 33.98% and 33.80%, respectively), compared to the SD group (SD-n - 38.73% and 38.31%, respectively). Conclusion: The pharmacological efficacy of nimesulide in the experimental model of acute inflammation induced by CAR is reduced in Wistar rats fed a WD. **Financial support**: CAPES and CNPq. References: Kabaran, S. J Health Popul Nutr., v. 33, n. 14, 2015. Beauchamp, G.K. Digestion, v. 3 (Suppl), n. 1-6, 2011.

04.063 Effect of 17-beta estradiol and of the selective estrogen receptor modulator (SERM) tamoxifen, on neutrophil migration in mice with zymosan-induced arthritis. Silva LA, Alves JC, Souza EV, Ferreira RB, Grespan R UFS – Ciências Fisiológicas

Introduction: This study was designed to investigate the effect of 17-β-estradiol on the migration of leukocytes into the articular cavity of knee joint in zymosan-induced arthritis in mice. Previous studies demonstrated the protector activity of estradiol in experimental models of arthritis, however, such effect has not been studied in this model. In addition, the effect of tamoxifen citrate, a selective estrogen receptor modulator (SERM) was also studied in this experimental model. **Methods:** Female Swiss mice were ovariectomized through bilateral surgery with dorsal incision. After two weeks, the animals were divided in two groups: group 1 received hormone replacement therapy during 5 days with 17-β-estradiol (100 ug / kg, s.c.) and group 2 received vehicle (sesame oil). After treatment, zymosan A (Sigma, St. Louis, MO, USA) 100ug / cavity in 20 ul sterile saline was injected intra-articularly. Concomitant, intact mice, without ovariectomy, received zimosan A in the right knee joint (positive control) and vehicle (saline) in the left knee joint (negative control). Others groups received tamoxifen citrate salt (Sigma, St. Louis, MO, USA) at doses of 0.1, 0.3 and 0.9 mg / mouse, s.c., for 5 days before arthritis induction. The control group received vehicle (sesame oil). On the fifth day, one hour after the last dose, arthritis was induced by zymosan as mentioned above. Four hours later, to evaluate leukocyte migration, the articular cavities of knee joints were washed twice with 5 µl PBS / EDTA and diluted to a final volume of 100 µl. Total cell counts and differential cell counts were performed, stained with Rosenfeld's stain and results were expressed as mean of neutrophils per cavity ± SEM. The means were compared by ANOVA followed by Tukey's post hoc test for multiple group comparisons ($p \leq 0.05$). **Results:** The group 2 of ovariectomized mice without hormone treatment showed an increase of neutrophils (22.71 ± 3.414) into the intra-articular cavity compared to the intact mice, without ovariectomy that received zimosan A in the right knee joint (15.76 ± 2.291, positive control). The group 1 with replacement of 17-beta estradiol showed a significant decrease in neutrophils (11.19 ± 2.246) when compared with group 2 (22.71 ± 3.414). There was no difference in neutrophils count between the groups treated with tamoxifen citrate salt at doses of 0.1 and 0.3 mg / mouse, but both showed a tendency in increase of neutrophils compared to the positive control. The group treated with a dose of 0.9 mg /mouse, showed a significant decrease in neuthophils (7.938 ± 1.542) when compared to the positive control (14.83 ± 1.998). **Conclusion**: These findings suggest that estrogen has anti-inflammatory activity, reducing migration of leukocytes into the intra-articular cavity in experimental model of zymosan-induced arthritis. Moreover, it is inferred that tamoxifen citrate salt at a dose of 0.9 mg / mouse estrogen exerts modulating activity, presumably acting as an agonist of estrogen receptors. Financial Support: CNPq. Research approval by the Animal Research Ethical Committee: Protocol 10/2015.

04.064 Nebulized gold nanoparticles down-regulates inflammation, mucus exacerbation and lung remodeling in a murine model of steroid-resistant asthma. Serra MF[1], Pimentel AS[1], Cotias AC[1], Lanzetti M[1], Hickmann J[2], Arantes ACS[1], Silva PMR[1], Cordeiro RSB[1], Barreto E[2], Martins MA[1] [1]Fiocruz – Fisiologia e Farmacodinâmica, [2]UFAL

Introduction: The reduced responsiveness to anti-inflammatory effects of glucocorticoids (GCs) is a significant barrier to an effective therapeutic management of severe asthma. Gold-based compounds have a long history of therapeutic use for the treatment of chronic inflammation due to its anti-inflammatory and anti-oxidant properties. We recently reported that nasal-instilled gold nanoparticles (AuNPs) prevented central features of asthma in short-term models of this disease. Here, we sought to determine the effectiveness of aerosolized gold nanoparticles in a long-term murine model of steroid-resistant asthma. **Methods:** Mice of strain A/J were subcutaneously sensitized at days 0 and 14 by a suspension of $Al(OH)_3$ and ovalbumin (OVA), and challenged for 9 consecutive weeks, once a week, starting at day 19 post-sensitization. Three weeks after the beginning of OVA challenges, mice were subjected to daily interventional nebulizations (2.5 L/min, 30 min) of either 12 nm AuNPs (0.4 µg/mL) or budesonide (7.5 mg/mL) 1 h before challenge. Lung function, leukocyte infiltration, mucus exacerbation, extracellular matrix deposition, cytokine generation and oxidative stress were evaluated 24 h after the last challenge (CEUA license # L-030/15). **Results:** We found that Ova-challenged mice developed marked AHR, lung eosinophil and neutrophil infiltrations, and increased peribronchial fibrosis and mucus production as compared to sham-challenged mice. All these changes were inhibited in mice treated with AuNPs, but not budesonide. Similarly, increased lung tissue levels of IL-4, IL-13, IL-17, eotaxin-1, eotaxin-2, KC and TARC appeared reduced after nebulized AuNPs, but remained unaltered following budesonide in this model. Furthermore, AuNPs treatment decreased the levels of ROS (16,4 ± 1,5 to 5,4 ± 1,4 µg formazan/10^6 cells, Mean ± SEM, n=7) and TBARs (2,3 ± 0,2 to 1,3 ± 0,1 nMol/mg protein - Mean ± SEM, n=7) and restored catalase baseline levels. In contrast, budesonide did not interfere with ROS, TBARS or antioxidant enzyme activities. **Conclusion:** We show that aerosolized AuNPs inhibits AHR, eosinophilic and neutrophilic lung inflammation, and airway remodeling and mucus exacerbation in a murine model of asthma, which expresses a marked refractoriness to glucocorticoid treatment. The protective effects of AuNPs treatment correlates anti-inflammatory properties and effectiveness in combating oxidative stress imbalance. Taking together, these results suggest that AuNPs should be further investigated as a therapeutic alternative for controlling difficult-to-treat asthma.
Financial Support: CNPq, FAPERJ and CAPES.

04.065 Arginase 1 importantly contributes to lung fibrogenesis in silicotic Swiss-Webster mice. Correa AMC, Dias DF, Ferreira TPT, Ciambarella BT, Arantes ACS, Martins MA, Martins PMRS [1]Fiocruz

Introduction: Inhalation of silica particle induces an inflammatory lung disease named silicosis, which is mainly characterized by an intense fibrosis and granuloma formation. Arginase 1 is an enzyme considered to be involved in fibrogenic responses and is associated with the presence of alternative macrophage phenotype (M2). **Aim**: This study was undertaken to investigate the potential contribution of arginase 1 to fibrosis and granuloma formation in the experimental model of silicosis in mice. **Methods:** Male Swiss-Webster mice were anesthetized and then instilled intranasally with crystalline silica particles (10 mg/50 µL) or saline (control), and the analyzes made 7, 14 and 28 days after silica provocation. The parameters included lung tissue morphology/morphometry and ii) identification of F4/80 positive cells and arginase 1 expression by immunohistochemistry. Animals were administered daily with the arginase 1 inhibitor Nor-NOHA (2.5 mg/kg, ip) during 7 days, starting 21 days after stimulation with silica and analyzes made 1 day after the last dose. All experimental procedures were performed in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (LW57/14). **Results:** We found that silica instillation led to morphological changes in the lung tissue, which reflected the presence of an intense inflammatory cell infiltrate followed by progressive fibrogenesis and marked granuloma formation. In another set of experiments we detected, by immunohistochemistry technique, an increase in the presence of F4/80 positive cells in the lungs of silicotic mice. Also, higher labeling for arginase 1 was noted in silicotic mice, suggesting the presence of the alternative macrophage phenotype (M2). In addition, when silica-challenged mice were given the arginase 1 inhibitor Nor-NOHA, therapeutically, a significant decreased of the granuloma area was shown. **Conclusion:** Altogether, our findings show that mice challenged with silica exhibit marked granulomatous fibrosis, which parallel with increased F4/80 positive cell population and arginase 1 in the lung tissue. All responses were sensitive to the arginase 1 inhibitor Nor-NOHA. This seems to be an indicative that arginase 1 seems to be a potential pharmacological target for the treatment of fibrotic diseases such as silicosis. **Financial Support**: FIOCRUZ, CNPq and FAPERJ.

04.066 Role of oxidative stress and intestinal microbiota in the pathogenesis of experimental steatohepatitis induced by irinotecan. Muniz HA[1], Aragão KS[1], Almeida PRC[2], Melo AT[1], Costa ML[1], Lopes CDH[1], Carvalho CBM[3], Ribeiro RA[1], Lima-Júnior RCP[1] [1]UFC – Physiology and Pharmacology, [2]UFC, [3]UFC – Medical Microbiology

Introduction: The nonalcoholic steatohepatitis (NASH) is a adverse effect of the anticancer agent irinotecan. NASH may worsen the clinical course of patients submitted to hepatic resection of metastatic colorectal cancer. Recently, we developed a new experimental animal model of the IRI-induced NASH. Here, we aimed to investigate the participation of oxidative stress and intestinal microbiota in IRI-induced NASH pathogenesis. **Methods**: Swiss mice (n=8, 25g) were divided into groups and injected saline (5 ml/kg, i.p.) or irinotecan (50 mg/kg, ip, 3x/week/7weeks). After 7 weeks, the livers were removed for measurement of malonaldehyde (MDA) and nonproteic sulfidryl groups (NPSH), and immunohistochemical (IHC) assay for iNOS. To investigate the participation of intestinal microbiota in NASH we used antibiotics association (ANTB). C57BL/6 mice (n=8, 25g) were injected with saline (5 ml/kg,ip), ANTB in the drinking water (50 mg/kg/day/7 weeks). Microbiota depletion was confirmed by culture of stool samples in BHI medium after 10 days of ANTB treatment. On day 10, IRI treatment was initiated. Peripheral blood samples were collected for measuring serum ALT (U/L) and bacteremia. Animal livers were used for histopathology by the Kleiner's scores (lobular inflammation [0-3], steatosis [0-3] and vacuolization [0-3]), IL-1β and TNF-α levels, lipid content, and also for IHC assay. ANOVA/Student Newman Keul or Kruskal Wallis/Dunn tests were used for statistical comparison. $P<0.05$ was accepted. **Results**: IRI increased MDA (408.3 ± 67.5) and decreased NPSH levels (361.3 ± 104.7) *vs* control group (MDA: 109.8 ± 6.3; NPSH: 551.5 ± 36.4). IRI also reduced body weight (36%), increased the serum ALT (25.4 ± 3.2 *vs* 17.3 ± 5.2), and liver wet weight (1.962 ± 165.3 *vs* 1.425 ± 39.5), local production of IL-1β (977.8 ± 55.6 *vs* 624.9 ± 49), TNF-α (1775 ± 76.7 *vs* 1434.8 ± 187.6) and lipid content (49.4 ± 2.5 *vs* 34.6 ± 1.9), and increased Kleiner's scores [5(3–7) *vs* 2(1–3)]. Bacteremia was evidenced in IRI-injected group (portal blood-80%) and (systemic blood-40%) versus saline group (0%), shortening of the villi and alteration of crypt architecture (12[8-17] *vs* 2,5[2-3]). Intestinal bacterial depletion was observed over the experimental period in ANTB treated animals. Interestingly, ANTB prevented ($p<0.05$) the loss of body weight, the increase of ALT (15.8 ± 1.5), liver wet weight (1,366 ± 74.3), tissue production of IL-1 (748.2 ± 28.9), TNF-α (1565 ± 27.7), lipids (37 ± 3.6), and also reduced Kleiner's scores (3[1–7]) *vs.* IRI group. In addition, ANTB therapy abolished irinotecan-related bacteremia (0%) versus IRI group. IRI group showed a significant increase in liver immunoexpression of iNOS (3[2–3] and TLR4 (2[0-3] *vs*) versus saline group (iNOS: 2[1–2]; TLR4: 0[0–1]). ANTB reduced the immunoexpression of these markers (iNOS: 1.5[1–3]; TLR4: 0[0-2]). In addition, TLR4 immunoexpression was increased in intestinal samples (IRI: 1[0–2] *vs* control: 0[0–0]), which was prevented by ANTB (TLR4: 0[0-1]). **Conclusion**: These results suggest that IRI causes rupture of gut barrier, leading to intestinal bacterial translocation to the liver and promoting NASH. Liver damage seems to be dependent on inflammatory cytokines and oxidative stress. **Support**: CNPq. **Animal research ethics committee:** 21/2012.

04.067 Warifteine, an alkaloid of cissampelos sympodialis, inhibits histological parameters in an allergic rhinitis model. Pereira RF[1], Gadelha FAAF[1], Paiva-ferreira LKD[1], Vieira GC[1], Bozza PT[2], Piuvezam MR[1] [1]UFPB, [2]Fiocruz

Introduction: Warifteine (WAR) is a chemical marker alkaloid isolated from the *Cissampelos sympodialis* Eichl (Menispermaceae), a plant used in Northeastern Brazil to treat respiratory disease. Previous studies with extractand its compounds, demonstrated anti-inflammatory and anti-allergic activities on asthma model. Allergic rhinitis (AR) is a chronic inflammatory disorder of the nasal tissue and, about one billion people worldwide is commented by this disease that affect their quality of life and it is a risk factor for asthma exacerbation. The aim of this study was to evaluate the effect of intranasal administration of WAR in an allergic rhinitis model induced by ovalbumin (OVA). **Methods:** The BALB/c mice (n=5) were sensitized on days 1th and 7th by i.p. injection OVA (50 µg) in aluminium hydroxide (5 mg). A week late, mice were challenged by nasal instillation of OVA (50 µg) on three successive days, for three consecutive weeks (between day 14th to day 35th). Two weeks later, mice were re-challenged seven times (from day 49th to day 55th) with OVA and 1h before each challenge the animals were treated with dexamethasone (DEX,2 mg/kg) or WAR (2 mg/kg).Twenty four hours after the last challenge on day 56th, the animals were euthanized and the heads were scissored treat by histological process and stained with hematoxylin-eosin (H&E), periodic acid-Schiff (PAS) or toluidine blue. **Results:** The inhaled treatment with WAR or DEX decreased cell infiltration into the perivascular regions (6.2 ± 3.8)(10.3 ± 2.9) respectively and the epithelium membranes were intact and thinner than that head material from OVA group (41.3 ± 3.0). In addition, these treatments reduced the mucus production(7.6 ± 3.1) (20.7 ± 2.9) as compared with head material from OVA group (39.1 ± 2.4) without modification of the number of mast cells (25.6 ± 4.9) on the nasal tissue. **Conclusion:** These results demonstrate for the first time that intranasal warifteine treatment down regulated histological parameters associated with allergic rhinitis. **References:** VIEIRA, G. C, Phytother Res, v. 13, p.1, 2013; M. WANG, Clin and Exper Allergy, v.43, p.956, 2013. **Financial support:** INCT-CANCER/FAPERJ/CNPq; CNPq-Universal14/2012-472853/2012-0; CAPES.Animal Research Ethical Committee:1805/14.

04.068 Effect of 1,4- cineol in acute lung injury model. Gadelha FAAF, Leite FC, Pereira RF, Vieira GC, Piuvezam MR UFPB

Introduction: 1,4-cineol is a monoterpene of essential oil of some medicinal plants. Pharmacological studies by our group with 1,4- cineol showed anxiolytic activity. The acute lung injury (ALI) is an acute inflammatory lung reaction with parenchyma injuries progressing to acute respiratory distress syndrome. The aim of this study was to investigate the possible anti-inflammatory effect of 1,4-cineol on acute lung injury model. **Methods:** BALB/c mice (n = 7) were treated with 1,4- cineol (100 mg/kg) dexamethasone (2 mg/kg) or vehicle (i.p.) 1 hour before lipopolysaccharide (LPS, 1 mg/kg) or PBS challenges. In addition, the treatments were also performed 24 and 48 h after LPS challenge. After this period animals were anesthetized for lung tissue collection to be processed for histological analyses with hematoxylin-eosin (H&E) stain. **Results:** Administration of 1,4- cineol (17.9 ± 7.6) effectively reduced inflammatory cell migration to the lung parenchyma and also inhibited hemorrhage (15.1 ± 7.6) in the airspace when compared to lungs from non-treated LPS-challenge animals (42.3 ± 2.6) (34.9 ± 3.5).It was also observed that 1,4- cineol promoted reduction of diffuse alveolar damage and alveolar collapse, however it was observed the presence of hyaline membranes. The dexamethasone treatment (15.1 ± 7.6) (12.3 ± 6.2) (15.1 ± 7.6) showed reduction of all parameters analyzed andan improvement in histopathological aspects of the lungs when compared with material from animals of the LPS. **Conclusion:** These results demonstrated that 1,4-cineol was able to ameliorate the histopathological parameters associated with acute lung injury. **References:** SHAH, D, Am J Physiol Lung Cell Mol Physiol, V. 306, p. 152, 2014. **Financial support:** INCT-CANCER/FAPERJ/CNPq; CNPq-Universal14/2012-472853/2012-0; CAPES. Animal Research Ethical Committee Nº 1211/11

04.069 Involvement of hormonal imbalance and epigenetic alterations in development of allergic lung inflammation, in low birth weight rats. Ramos APA[1], Balbino AM[2], Gil NL[1], Azevedo GA[1], Arakaki CP[1], Carvalho MHC[3], Landgraf RG[1], Landgraf MA[1,3,4] [1]Unifesp-Diadema – Ciências Farmacêuticas, [2]UNIFESP – Ciências Farmacêuticas, [3]USP – Farmacologia, [4]Unip

Introduction: It has been demonstrated by our group that rats with low birth weight due to maternal malnutrition during pregnancy showed attenuation in allergic inflammatory response. Here, we evaluated the involvement of hormones and lung global methylation pattern in the development of allergic lung inflammation. **Methods:** Pregnant female Wistar rats were randomly divided into two groups: nourished ad libitum and undernourished. Nourished female rats were fed a standard commercial rat diet, whereas undernourished female rats were fed the same diet at 50% of the nourished female rats intake. 9-week-old male rats from the undernurished (low birth weight offspring – LBW) and nourished mothers (NR) groups were sensitized on days 0 and 7 by an intraperitoneal injection containing 50 µg of ovalbumin (OVA) and 1 mg of Al (OH)3; then, both groups were challenged with 2,5% nebulized OVA for 20 minutes on days 14 and 21. The control group consisted of rats immunized as previously described and challenged with phosphate buffered saline (PBS) solution. Rats were euthanized at 24 h after the final OVA challenge, and cell counts in bronchoalveolar lavage (BAL) fluid and peribronchial tissue were analyzed. Lung global methylation pattern and prostaglandin E_2 (PGE_2) levels were determinated, in lung tissue; ACTH, leptin and corticosterone levels were evaluated, in serum. **Results**: The increase in cell infiltration, in BAL, and in lung tissue following immunization and challenge were blunted in the LBW rats compared to the NR rats. LBW rats also exhibited lower PGE_2 levels than NR rats. LBW already presented high levels of corticosterone in basal conditions, and were not modified by antigen challenge. Different from observed in LBW, NR presented reduction in ACTH levels after OVA stimuli. There is no difference in basal leptin levels, when compared LBW to NR group; however OVA challenge enhanced leptina production only in NR group. Global lung methylation were significantly higher in LWB, compared to NR group. **Conclusion:** Our preliminary results indicate that epigenetic alterations and failure to regulate hormones, such as ACTH, corticosterone and leptin, might be involved in attenuated allergic lung inflammation presented by LBW rats. **Financial support:** FAPESP (2012/51104-8, 2010/01404-0, 2015/23026-0) and CNPq. **Animal Research Ethical Committee:** CEUA 4170110316.

04.070 Peripheral efficacy of resolution factors in the carrageenan-induced paw edema and hyperalgesia models in rats: a comparison between resolvin E1 and D1. Fonseca FCS[1], Orlando RM[2], Augusti R[2], Turchetti-Maia RMM[1], Francischi JN[1] [1]UFMG – Farmacologia, [2]UFMG – Química

Introduction: The discovery of Resolution Factors (RF), the lipids Resolvins, Protectins and Maresins (SERHAN *et al*., 2015), opened new possibilities for both the understanding of the pathophysiology and the therapeutics of the inflammatory process. The aim of the present work was to compare the profile of anti-inflammatory and analgesic activities presented by Resolvins E1 (RvE1) and D1 (RvD1) administered locally to a rat paw inflamed by carrageenan. **Methods**: The hind paws of male Holtzman rats (150-180 g) were pre-treated subcutaneously with either RvE1 (285 pmoles) or RvD1 (570 pmoles) diluted in 0.1 mL of an ethanol/saline solution (2-5%; Veh), 10 minutes before the injection of carrageenan (CG=100 or 500 µg, in 0.1 mL saline = zero time). Control groups received the Veh in the paws in place of Resolvins. Paw edema (Δ increase in volume, in ml) and mechanical hyperalgesia (Δ thereshold, in g) were measured with an Ugo Basile plethysmometer and the Randall-Selitto analgesimeter, respectively, at 0, ¼, ½ , 1, 2, 3, 4, 6 and 24 h. Chemical structures of Resolvins were confirmed by HPLC (ARITA *et al.*, 2006). Means$\pm$sem were calculated at each time point and differences compared using two-way ANOVA, with Bonferroni posthoc analysis, being considered significant when $P<0.05$. **Results**: Previous treatment using RvE1 reduced paw hyperalgesia ($RvE1+CG_{500}=-22\pm15.9$; $Veh+CG_{500}=-50\pm8.4$) due to both doses of CG but only the edema of the lower dose CG ($RvE1+CG_{100}=0.64\pm0.04$; $Veh+CG_{100}=0.99\pm0.02$) at its maximum effect (2 or 3h). Moreover, single administration of Rvs 10 min before the maximum effect only reduced hyperalgesia due to the higher dose CG (CG_{500}+RvE1=-52$\pm$5.83; CG_{500}+Veh=-82$\pm$5.83). In addition, local administration of both Rvs simultaneously didn´t reduced further the anti-inflammatory and analgesic effects compared with either dose individually. **Conclusions**: 1. Resolvins were effective anti-inflammatory and analgesic agents given peripherally *before* the proinflammatory stimulus, being RvE1 2-fold more potent than RvD1; 2. Analgesic, but not the anti-inflammatory activity, remained detectable when either Resolvin was given to an on-going inflammation; 3. RvE1 and D1 seem to show a vocation to be analgesic rather than anti-inflammatory compounds. **References**: 1. SERHAN, C. N.; DALLI, J.; COLAS, R. A.; WINKLER, J. W.; CHIANG, N. Review: Protectins and maresins: New pro-resolving families of mediators in acute inflammation and resolution bioactive metabolome. Biochim. Biophys. Acta. v. 1851, p. 397–413, 2015. 2. ARITA, M.; OH, S. F.; CHONAN, T.; HONG, S.; ELANGOVAN, S.; SUN, Y; UDDIN, J.; PETASIS, N. A.; SERHAN, C. N. Metabolic Inactivation of Resolvin E1 and Stabilization of Its Anti-inflammatory Actions. J. Biol. Chem. v. 281, n. 32, p. 22847–22854, 2006. **Financial support**: CNPq, CAPES and FAPEMIG Experimental procedures were approved by the local Animal Ethics Committee (CEUA/UFMG, n#199/2014).

04.071 Acute lung inflammation induced by intestinal ischemia and reperfusion is influenced by ovariectomy in obese mice. Rodrigues-Garbin S[1], Fantozzi ET[1], Ricardo-da-Silva FY[1], Oliveira-Filho RM[1], Riffo-Vasquez Y[2], Tavares-de-Lima W[1] [1]ICB-USP, [2]Sackler Institute – Kings College

Introduction: More than half million people in the world are obese. Some studies indicated that obesity correlates with a worse prognosis of lung inflammation caused by ischemic episodes. Acute lung inflammation (ALI) is a common complication caused by intestinal ischemia and reperfusion (IR) and female sex hormones (FSH), notably estradiol, modulates this inflammation. In this study, we evaluated the role of FSH on the ALI in obese mice after the IR. **Methods:** Female mice C57/Bl6 (21 days; 20g) with the intact ovaries (non-OVx) and ovariectomized (OVx-9 weeks) were fed (9 weeks) a high fat diet (HFD). After the 9 weeks, they were subjected to IR by occlusion of the superior mesenteric artery (45 min) followed by 2 h of reperfusion. Control groups consisted of non-OVx mice and OVx mice. After 2 h of reperfusion, the mice were euthanized and the lungs were removed to quantify the microvascular permeability (Evans blue dye extravasation, EB), mobilization/activation of neutrophils (mieloperoxidase assay, MPO) and cytokines levels in supernatant of lung explant (magnetic luminex screening assay). Blood granulocytes and bone marrow cells (BMC) were also quantified (hematological analyzer and optical microscopy, respectively). Statistical analyses were performed comparing 2 groups using Student's t test. **Results:** OVx/HFD group of mice increased EB dye, MPO activity in the lungs and circulating granulocytes. After the IR, OVx/HFD mice showed an intensified increase of EB dye, granulocytes and in bone marrow cells whereas MPO activity was not modified. Non-OVx/HFD after IR increased lung MPO activity reduced the number of granulocytes and did not change the bone marrow cells. Lung of OVx/HFD mice released low levels of TNF-α, MIP-2, leptin, IL-17, IL-1β relative to Non-OVx/HFD. When IR was induced in OVx/HFD only VEGF and IL-1β in the lung tissue were elevated in comparison to Non-OVx/HFD submitted to IR. Regarding the Non-OVx/HFD after IR we found a reduced level of VEGF in relative to Non-OVx/HFD. **Conclusion**: Our results suggest an interaction of long-lasting effects of ovariectomy with obesity in order to reduce the lung release of cytokines but also, involve a mobilization of granulocytes that may migrate into the lung and contribute to increase the microvascular permeability. This interaction interfered with the IR exacerbating the increase of microvascular permeability, the mobilization of blood granulocytes and bone marrow cells as well as the release of VEGF IL-1β by the lung. *Financial Support: FAPESP* (2012/50550-4) *CAPES, CNPq,* Ethics Committee number: 076/2015/CEUA – ICB/USP

04.072 Investigation of the anti-inflammatory activity of N-salicyloyltryptamine on carrageenan-induced peritonitis in Mus musculus. Sousa Neto BP, Gomes BS, Everton SS, Macêdo FVC, Arcanjo DDR, Gutierrez SJC, Oliveira FA – UFPI – Farmacologia

Introduction: The indole nucleus core is one of the most natural bioactive chemical structures, and is part of many natural and synthetic molecules. The N-salicyloyltryptamine (NST) is an indole-derived compound which possesses anticonvulsant, muscle relaxant and hypnotic properties. The present study aims to determinate the NST-induced anti-inflammatory activity on the carrageenan-induced peritonitis model in mice. The total leukocytes were counted, as well as the total protein concentration, the activities of myeloperoxidase (MPO) and catalase (CAT), the nitrite concentration (NO_2^-), and thiobarbituric acid reactive species (TBARS) were assessed. **Methods**: Male Swiss mice (n=6/group) were intraperitoneally treated with vehicle, NST (50, 100 or 200 mg/kg), or indomethacin (10 mg/kg). After 30 min, they were intraperitoneally treated with 0.05 mL of carrageenan (1.0%). Then, the animals were euthanized after 4 h, the peritoneum was washed with 5.0 mL of heparin solution in PBS (10 UI/mL). The total leukocyte count was performed using 20 µL of exsudate and 380 µL of Turk solution in a Neubauer chamber. After centrifugation of exsudate, the analysis of total proteins was performed using 500 µL of supernatant in a biochemical analyzer (Labtest®). The MPO was analyzed in 100 µL of supernatant added to 1000 µL of 0.5% HTAB buffer. After centrifugation, 10 µL of supernatant was added to 200 µL of reading solution, and analyzed at 450 nm. The determination of CAT activity was performed by adding 200 µL of exsudate to 1200 µL of 50 mM PBS (pH 7.0), and then to 1000 µL of 30 mM H_2O_2 solution, and the absorbance was measured at 240 nm. In order to assess the NO_2^- content, 200 µL of exsudate was diluted in distilled water, and then deproteinized. Afterwards, 100 µL of supernatant was added to a 96-well plate, and then 100 µL of Griess' reagent was added. After 10 min, the absorbance was readed at 550 nm. The TBARS was measured in 200 µL of exsudate added to 350 µL of 20% acetic acid, and 600 µL of 0.5% thiobarbituric acid (TBA). The samples were boiled during 45 min, followed by ice bath during 15 min. Then, 50 µL of 8.1% SDS was added and the samples were centrifuged. The absorbances were readed at 420, 490, and 550 nm. **Results**: The intraperitoneal pretreatment with NST (100 and 200 mg/kg) decrease significantly (***$p<0.001$) the migration of total leukocytes, and the concentration of total proteins, when compared with vehicle. Interestingly, the pretreatment with NST, at doses of 100 and 200 mg/kg, decrease significantly the MPO (64.32% and 62.95%, respectively), and CAT (49.35% and 61.58%, respectively) content, when compared with vehicle (**$p<0.01$). Furthermore, the pretreatment with NST (100 e 200 mg/kg) promoted decrease in the nitrite (56.20% and 60.60%, respectively; ***$p<0.001$), and the TBARS content (33.76% and 48.71%, respectively; **$p<0.01$). The positive control indomethacin decrease significantly all the parameters assessed (*$p<0.05$). Conclusion: These findings indicate the NST affect the inflammatory mediators involved in the pathogenesis of the carrageenan-induced peritonitis, as well as suggests these actions might be responsible for the anti-inflammatory effect induced by this indole-derived compound. **Financial Support**: UFPI / CAPES. (CEEA/UFPI, permssion Nº 082/14).

04.073 Chemical and surgical models of temporomandibular osteoarthritis display distinct patterns of local inflammation in rats. Togni L[1,2], Abreu MC[1], Silva RB[1,3], Campos MM[1,2,3] [1]INTOX-PUCRS – Toxicologia Pré-clínica, [2]PUCRS – Patologia, [3]PUCRS – Medicina e Ciências da Saúde

Introduction: Anterior disc displacement (ADD) is an internal derangement frequently observed in the temporomandibular joint (TMJ) of patients with temporomandibular disorders (TMD). Evidence suggests a strong connection between ADD and osteoarthritis (OA) development (Bertram et al, J Oral Rehabil, 39:93, 2012). Most animal models of TMJ-OA induction are limited to an intra-articular infiltration of phlogistic agents or an occlusal abrupt alteration in rodents; however, no rat model exhibits OA induction subsequent to ADD (Hui et al, J Craniofac Surg, 25:2112, 2014; Zhang et al, J Dent Res, 92:253, 2013). The purpose of this study was to compare a well-established model of OA induced by an intra-articular infiltration of complete Freund's adjuvant (CFA), with a surgical model of OA development following ADD in the rat TMJ. **Methods:** Male Wistar rats (160-180 g; n=8/group) were randomly divided into two surgical groups, namely ADD and sham-operated. Two additional experimental groups received an intra-articular infiltration of CFA or saline solution (NaCl 0.9 %). Different experimental subgroups were euthanized at 15 or 30 days after procedures, and the region corresponding to TMJ was collected for further histological analysis, by hematoxylin-eosin (H&E) staining. The fibrocartilage thickness was measured (in µm), in the anterior, middle and posterior thirds of the condyle surface. Data were analyzed by one-way ANOVA followed by Bonferroni's test. **Results:** At 15 days, the fibrocartilage thickness was significantly increased in middle and posterior condyle portions in the ADD group (middle, 446 ± 88µm; posterior, 373 ± 37µm), when compared to sham-operated animals (middle, 246 ± 8µm; posterior: 245 ± 18µm). The CFA-injected animals displayed significantly augmented thickness only at the posterior region (372 ± 43µm), in relation to saline-injected control group (174 ± 7µm). At 30 days, there was a marked increment of the fibrocartilage thickness in the anterior third of the condyle in the ADD group (ADD, 375 ± 96µm; sham-operated, 152 ± 6µm). At this time-point, no significant change was detected in the CFA group, regardless of the analyzed region (CFA, 139 ± 13µm; saline, 154 ± 6µm). **Discussion:** This study describes a novel model to study TMJ-OA development in rats, featuring the anatomical alterations seen in the clinical set. Further studies are in progress to better characterizing the differences between the ADD and the CFA model, both locally and systemically. ADD might well represent a useful rat model to assess new strategies to manage TMJ-OA. **Financial support:** CAPES, CNPq, FINEP, PUCRS. **Animal Ethics Committee approval:** Pontifícia Universidade Católica do Rio Grande do Sul (CEUA 15/00465).

04.074 Sulphoraphane modulates joint inflammation in CFA-induced mono-arthritis. Rodrigues JFS, Silva CS, Muniz TF, Nina LNS, da Silva LCN, Fernandes ES, Grisotto MAG Ceuma

Introduction: Rheumatoid arthritis (RA) is defined as the inflammation of one or more joints that affects about 1% of the adult world population (Valdes & Spector., 2008). Its main features comprise the presence of severe inflammation and hardening of the joints after prolonged rest (Manzo et al., 2010). RA treatment includes non-steroidal anti-inflammatory drugs, disease modifying agents and biologicals. Although therapy has improved the quality of life of patients by attenuating pain, it does not halt disease progression. Thus, there is a real need to develop new drugs to treat RA. Sulforaphane (SFN) is a natural compound found in cruciferous vegetables such as broccoli, cabbage and Brussels sprouts which has been pointed as a potent anti-oxidant (Dinkova-Kostova and Talalay, 2008). Here, we investigated the effects of SFN in a mono-arthritis model induced by CFA in mice. **Methods:** Mono-arthritis was induced in female and male C57BL/6 mice (6-8 weeks old) by a single intra-articular injection of CFA (10 µg/10 µl) into the ipsilateral knee joint and 10 µl of saline into the contralateral joint as described by Fernandes et al. (2011). From day 4^{th} post mono-arthritis induction, mice were treated with SFN (10 mg/kg, i.p) twice a day for 3 days. Vehicle (3% DMSO in saline)-treated mice were used as controls. Mechanical allodynia and hyperalgesia were evaluated by using Von Frey Filaments (0.4g and 0.6g, respectively). Knee joint thickness was measured by calipers and taken as indicative of oedema formation. Synovial fluid thioredoxin reductase levels were evaluated by using an Enzyme-Linked Immunosorbent Assay (ELISA) kit. Synovial fluid cell populations were evaluated by flow cytometry. **Results:** As expected, CFA-injection in vehicle controls induced mechanical allodynia and hyperalgesia; in addition to joint oedema in comparison with PBS-treated joints. SFN had no effects in either mechanical allodynia or hyperalgesia. However, the same treatment reduced CFA-induced joint sweeling. Quantification of thioredoxin reductase showed that treatment with SFN induces an increase in enzyme activity in synovial fluid samples obtained from both CFA- and PBS-injected joints. Also, SFN-treated mice exhibited higher numbers of $Ly6G^+$ and $CD11b^+$ cells in the synovial fluid of CFA, but not PBS-injected joints in comparison with samples obtained from vehicle controls. **Conclusion:** Our results show that SFN increases the number of $Ly6G^+$ and $CD11b^+$ cells in the synovial fluid whilst reducing knee joint swelling. It is possible these cells play a role in the resolution of inflammation in our model. Overall, our data suggest that SFN may represent a novel therapy to treat joint inflammation, however further studies are necessary to clarify its mechanism of action. Financial Support: FAPEMA and CAPES This study was approved by the Animal Ethics Committee (CEUA -protocol 245/14). Dinkova-Kostova A.T, Talalay P. Mol Nutr Food Res.**;** 52: S128-38, 2008. Fernandes E.S, et AL. Arthritis Rheum. V63; 819-29; 2011. Manzo A, Bombardieri M, Humby F, et al. Immunol Rev.V85; 233:267; 2010. Valdes AM, Spector TD**.** Rheum. Dis. Clin. North Am. V34: 581–603, 2008.

04.075 P-Coumaric acid protects against lipopolysaccharide-induced acute lung injury in mice by modulating inflammatory cells and cytokine production. Souza TNC, Ferro JNS, Silva LMP, Corrêa ACC, Santos FM, Júnior JCF, Conserva LM, Barreto EO [1]UFAL – Ciências Biológicas e da Saúde, [2]UFAL – Química e Biotecnologia

Introduction: Acute lung injury (ALI) is still a significant clinical problem with a high mortality rate and there are few effective therapies in clinic. Previous studies have reported that p-coumaric acid (CA) a natural phenolic compound, possesses relevant pharmacological properties including antioxidant effect and immunomodulatory activity. However, its effect on lung inflammation remains unknown. Therefore, we sought to evaluate the effect of p-coumaric acid on acute lung injury induced by lipopolysaccharide (LPS). **Methods**: Male Swiss mice (30 g) were intranasally exposed to CA (0.1 or 1 µg/Kg) 1 h prior LPS stimulation (10 µg/mice) for inducing ALI. At 12 h after LPS stimulation, bronchoalveolar lavage fluid (BAL) and lung tissues were collected, and the number of inflammatory cells was determined. Next, levels of interleukin-6 (IL-6), interleukin-8 (IL-8) and interleukin-10 (IL-10) in BAL were measured by ELISA assay. Pathological changes of lung tissues were observed by H&E staining. Another set of experiments, the adherence of neutrophils to vascular endothelial cells was evaluated in vitro. For this purpose, human neutrophils with or without p-coumaric acid pretreatment were allowed to adhere on TNF-alpha-stimulated human vascular endothelial cell line (EA.hy926 cells). After 1 h, non-adherent cells were washed away with phosphate-buffered saline, and the remaining cells were fixed in ethanol and stained with Giemsa. The number of adhered neutrophils per endothelial cell was determined by direct counting. The data were expressed as an index of adhesion (IA), which was calculated as follows: IA = [(EA.hy926 with bound neutrophils)/total EA.hy926 number] × [(neutrophils bound to neutrophils)/total neutrophils number] × 100. The two-way ANOVA followed by post hoc Turkey test was used for statistical evaluation between experimental groups with $P<0.05$ considered significant. **Results**: LPS stimulation increased total leukocyte counts ($p<0.01$) which was decreased by pretreatment with 0.1 and 1 µg/kg CA at 43% and 51%, respectively. In case of neutrophil count, the increased levels in BAL were reduced from $15.0x10^4$ cells to $6.3x10^4$ cells, and $4.8x10^4$ cells, following pretreatment with 0.1 and 1 µg/kg CA, respectively. In addition, treatment with 1 µg/kg CA decreases the amount of IL-6 to 70% and IL-8 to 83%, at the same time that markedly increased levels of IL-10 in 40% in BAL fluid. Compared with the LPS-stimulated group, the levels of inflammatory cell infiltration around the bronchi of the lung was significantly declined with pretreatment 0.1 and 1 µg/kg CA ($p<0.001$). In vitro studies revealed that pretreatment with p-coumaric acid was able to suppress the index of adhesion neutrophil to TNF-activated endothelial cells ($p<0.001$). Conclusion: These results show that CA, administered topically, can prevent LPS-induced lung injury. The protective effect of CA on acute inflammatory changes appears to be mediated by IL-10 production. Moreover, CA may prevent the interactions between neutrophils and endothelium. **Financial support**: CNPq, CAPES. Research approval: Animal Research Ethical Committee (Licence no. 67/2014)

04.076 Evaluation of anti-inflammatory activity of oleoresin of *Copaifera reticulata.* Almeida Junior JS[1], Silva EBS[1], Araujo JA[1], Sartoratto A[2], Moraes TMP[1], Oliveira ECP[1], Moraes WP[1]
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Introduction: "Copaíba", as *Copaifera reticulata* is popularly known, is a species widely spread throughout the Amazon region, and when its trunk is tapped an oleoresin is exuded. This oleoresin is widely used in traditional medicine, especially as anti-inflammatory, and therefore it is necessary to carry out studies that prove its effects described in ethnopharmacological work. This study evaluates thepharmacological effect of the oleoresin of *Copaifera reticulate*, aiming to corroborate the search for evidences to justify its use in traditional healing. Additionally, this study aims to determine the chemical composition of the oleoresin of *Copaifera reticulata* and evaluate its potential as an anti-inflammatory agent, using the Rat Air Pouch Model. **Methods:** The oleoresin used for the experiments was collected in the Tapajós National Forest (FLONA), in the municipality of Belterra-PA, in a dry period, and characterized chemically by a Gas Chromatograph coupled with Mass Spectrometry (GC-MS). In order to evaluate its anti-inflammatory activity, it was used the Air Pouch Method, which consists of administering sterile air in the interscapular region of the animal to produce a pouch. After inducing inflammation with carrageenan and offering itssubsequent treatment, it was collected the exudate formed in the pouch, measured its volume and quantified the number of inflammatory cells in it. The doses of the main mediators involved in the inflammatory process were measured, as follows: nitrite, TNF-α, IL-1β and prostaglandin E2. **Results:** The oleoresin of *Copaifera reticulate* presentedBeta-bisabolene as its major chemical component, which is a sesquiterpene already described in literature for its anti-inflammatory action. The oleoresin of *Copaifera reticulata* was able to reduce the formation of exudate, produced due to an inflammatory stimulus, and the number of inflammatory cells that migrated to the air pouch was significantly reduced. The levels of nitrite, a metabolite of nitric oxide, were also significantly reduced and in a dose-dependent manner. Another mediator that had its levels reduced in the pouch exudate was TNF-α, which is an important pro-inflammatory cytokine and is involved, for instance, in cell recruitment and increase of vascular permeability. The levels of IL-1β analyzed in the exudate presented a significant reduction. The most important prostanoid in the inflammatory process was also evaluated. The oleoresin under study reduced the formation of prostaglandin E2, which is a mediator involved in the formation of exudate and pain. **Conclusion:** In conclusion, the oleoresin *Copaifera reticulata* is composed of sesquiterpenes such as Beta-bisabolene, and demonstrated anti-inflammatory activity, reducing significantly the levels of the main mediators of the inflammatory response NO, PGE2, TNF-α and IL-1β, and also reduced the volume of exudates produced in the air pouch, as well as the recruitment of inflammatory cells. However, further studies are necessary to evaluate the mechanism of action of the oleoresin to inhibit the production of these mediators. **Key words:** Nitrite, TNF-α, IL-1β, Prostaglandin E2, Oleoresin of *Copaifera reticulata*. **Financial Suport:** Universidade Federal do Oeste do Pará **Number of approval by the Ethics Commitee of UniversidadeFederal do Oeste do Pará for use of animals:** Certificate number 07004/2013

04.077 Suppressive effects of oral quercetin administration on the late phase of experimental silicosis in mice. Guimarães FV, Ferreira TPT, Arantes ACS, Martins MA, Silva PMR Fiocruz

Introduction: Silicosis is a chronic occupational disease caused by inhalation of crystalline silica particles and is characterized by intense inflammation and fibrosis. In spite of the therapeutic arsenal currently available, there is no specific treatment for this disease. Quercetin is a flavonoid present in several plants including fruits, vegetables and some grains, which was shown to have important antioxidant and anti-inflammatory properties. **Aims**: In this study we evaluated the potential therapeutic effect of quercetin on the late phase of the experimental silicosis in mice. **Methods**: Male Swiss-Webster mice were instilled with silica intransally (10 mg/50 µL) and quercetin was administered orally (2.5 – 10 mg/kg), once a day, for 7 consecutive days, starting 21 days post-silica. N-acetylcysteine (150 mg/Kg), a classical anti-oxidant drug, was used for comparison. All analyses were carried out 24 h after the last dose of the compounds. Inflammatory, oxidative and fibrotic markers were measured both in *in vivo* and *in vitro* systems. All experimental procedures were performed in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (LW57/14). **Results**: We showed that quercetin, at the doses of 5 and 10 mg/Kg, led to a marked reduction of oxidative stress markers including reactive oxidative stress (ROS) production, malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity in the lungs of silica-challenged mice. The lipid peroxidation marker 8-isoprostane was attenuated by quercetin, though no effect was noted on catalase (CAT) enzymatic activity. In parallel we noted that quercetin also attenuated leukocyte infiltration, tissue fibrosis and granuloma formation as well as increased lung resistance and elastance and airway hyper-reactivity in the silicotic mice as compared to the controls. A similar inhibitory profile was noted after the administration of the classical anti-oxidative compound N-acetylcysteine (NAC) to mice instilled with silica. **Conclusion**: Our findings show that oral administration of quercetin reduced oxidative stress markers and inflammation as well as fibrosis in the lungs of silicotic mice. These findings indicate that quercetin seems to be a promising pharmacological tool for the treatment of inflammatory and fibrotic diseases such as silicosis. **Financial Support**: FIOCRUZ, CNPq, FAPERJ, CAPES and European Community (UE FP7- 2007-2013 - n°HEALTH-F4-2011-281608).

04.078 Nopharmacological treatment, using aerobic training and low intensity laser protect the articular capsule on experimental monoarthritis. Silva AD, Zamuner LF, Silva MP, Sanches IC, de Angelis K, Chavantes C, Zamuner SR Uninove

Introduction: Monoarthritis (MO) is disease that involves joint associated with pain, presence of inflammation and degeneration of intra-articular structures. This context affects the quality of life. **AIM:** Evaluate the combination of non-invasive therapies of aerobic training (AT) and low intensity laser therapy (LILT) in the deleterious alterations of the articular capsule. **Methods:** Male Wistar rats (n=38) were divided into sedentary control (C=6); monoarthritis (MO=8); MO + LILT (MOL=8), MO + AT (MOT=8) and MOT + LILT (MOTL=8). MO was induced by intra-articular injection of zymosan (1 mg/50 µL saline) into the right knee joint. Treatment protocol with moderate AT treadmill for 8 weeks (5 days/week) and LILT was applied at 660 nm (5mW power, power density of 0.1 W/cm^2 power density of 2.5 J/cm^2, 0.04 cm^2 spot area and irradiation time 20 sec) twice a week. Rats were euthanized after 56 days of treatment and the histology and cellularity was assessed in knee joints. **Results:** Rats subjected to combined treatment protocol (MOTL) showed preservation and improvement in functional capacity (MO, Inicial 12 ± 8.8 min, final 8.31 ± 8.31 min; MOTL, inicial 8.47 ± 7.9min, final 16.15 ± 17.5 min) ($p<0.0001$) and a decreased of leukocyte influx in the joint cavity (C, 162.5 ± 120 cellx10^4/ml; MO, 471.75 ± 277 cellx10^4/ml; MOL, 109 ± 22 cellx10^4/ml; MOT, 165.5 ± 122 cellx10^4/ml; MOTL, 81.2 ± 30.7 cellx10^4/ml) ($p=0.0037$). Histological analyses showed preserved morphology of synovial membrane and less collagen deposit (C, 3.43 ± 2.3 u.a/%; MO, 5.17 ± 2.1 u.a/%; MOL, 3.45 ± 2.4 u.a/%; MOT, 2.99 ± 2.3 u.a/%; MOTL, 2.05 ± 1.4 u.a/%), however, was not significant ($p=0.543$). **Conclusion:** MO promotes deleterious actions on the knees of rats. AT and LILT association have proved to be effective in knee joint protection, allowing preservation of functional capacity, intra-articular regulation and morphological organization of capsule.

04.079 Friedelin modulates intracellular redox status in epithelial cells *in vitro* exposed to cigarette smoke combined with LPS. Santos FM[1], Ferro JNS[1], Silva-Júnior AJ[1], Santos SL[1], Conserva LM[2], Broetto L[1], Barreto E[1] [1]ICBS-UFAL, [2]IQB-UFAL

Introduction: Oxidative stress has been implicated in the pathogenesis and progression of inflammatory airway diseases, and cigarette smoke is known to be one of the major sources of oxidants in the lungs. Natural plant-derived products are commonly applied to treat a broad range of human diseases, including airway inflammation. In this regard, friedelin, a known pentacyclic triterpene, exhibits diverse biological activities, which include anti-inflammatory and antioxidant actions. Despite the potentially significant health effects of friedelin, there are little information is available about their effects against oxidative stresses. Thus, the present study investigated the possible protective role of friedelin against oxidative stress induced by cigarette smoke along with LPS in epithelial cells. **Methods**: Cigarette smoke extract (CSE) was prepared as describe previously (Victoni, PlosOne, 9(1):e85243, 2014) by bubbling two Marlboro cigarettes (with filter, Philip Morris - Brazil) through 20 ml RPMI-1640 using a pump (15 ml/min), resulting in suspension considered to be 100% CSE. Then, the extract was filtered (0.22 µm membrane filter) and diluted to final concentration of 5% in RPMI-1640 medium. CSE (5%) containing LPS (1 µg/mL) was used to the cell stimulation. Human alveolar epithelial cell line (A549) was first treated with friedelin (1, 10 and 100 µM) for 4 h followed by exposure to CSE/LPS. After 24 h, intracellular reactive species (ROS) levels were measured by NBT assay, while mRNA expressions of superoxide dismutase 1 (SOD-1), catalase (CAT), glutathione-peroxidase (GSH-Px) and Nrf2 were assessed using RT-PCR. Additionally, after treatment with friedelin the cell viability was analyzed by MTT assay. The results are presented as mean±standard error of the mean. The two-way ANOVA followed by post hoc Tukey test was used for statistical evaluation between experimental groups with $P<0.05$ considered significant. **Results**: The exposure of A549 cells to CSE/LPS significantly increased intracellular ROS levels by approximately 127.39% when compared to the vehicle-treated cells. This ROS induction was reduced by pretreatment with friedelin at doses 1, 10 and 100 µM in 2%, 23.02% and 43.4%, respectively. The mRNA expression levels of CAT, GSH-Px, SOD and Nfr2 in A549 cells were significantly increased ($P<0.05$) after stimulus with CSE/LPS. Compared to the CSE/LPS-stimulated cells, A549 cells treated with 100 µM friedelin showed an upregulation in the levels of mRNA expression of CAT ($P<0.05$), HO-1 ($P<0.05$) and Nrf2 ($P<0.05$), but not of GSH-Px or SOD. Friedelin alone did not affect cell viability at all doses tested at 24h. **Conclusion**: Our results indicate that friedelin seems relieve oxidative stress via reduction of cellular ROS level and upregulate the gene expressions of antioxidant enzymes. **Financial support**: CNPq and CAPES.

04.080 Gold nanoparticles reduce pulmonary lung function and airway hyper-reactivity in silicotic in mice. Ribeiro NBS, Ciambarella BT, Arantes AC, Serra MF, Martins MA, Silva PMR
Fiocruz – Farmacologia e Inflamação

Introduction: Inhalation of crystalline silica particles leads to development of silicosis, an occupational disease, which is characterized by leukocyte infiltration, collagen deposition and granuloma formation. There is no efficient treatment available for fibrotic diseases, which demands the search for effective therapies to control silicosis. Remarkably, administration of gold nanoparticles (AuNPs) can lead to anti-inflammatory effects in different pathophysiological conditions. **Aims:** In this study we intended to investigate the effect of aerosolized AuNPs on lung function and granulomatous fibrosis trigerred by silica particles in mice. **Methods:** Anesthetized male Swiss-Webster mice received intranasal (i.n.) instillation of silica (10 mg/50 µL) or vehicle (saline). Treatment consisted of 3 aerosol administrations of AuNPs (1 - 10 µg/kg) on days 21, 24 and 27 after silica instillation. The analyses were made 24 h after the last administration and included the following parameters: i) lung function (resistance and elastance) and airways hyper-reactivity to metacholine (3-81 mg/mL) by invasive plethysmography (Finepointe, Buxco System); ii) morphological alterations analyzed by histological techniques including staining with Hematoxylin-Eosin and Picrus sirius; iii) quantification of tissue collagen content and TNF alpha done by Sircol and ELISA, respectively. All experimental procedures were approved by the Committee on Use of Laboratory Animals of Oswaldo Cruz Foundation (license LW 57/14). **Results:** Exposure of mice to silica particles yielded higher baseline lung resistance and elastance when compared to saline group. After aerosolization of the bronchoconstrictor methacholine, silica-challenged mice exhibited increased lung resistance and elastance. Therapeutical administration of AuNPs to silicotic mice inhibited alterations in the lung function and airways hyper-reactivity, though no effect was noted on collagen deposition and granuloma formation. TNF alpha generation in lung tissue was blocked by AuNPs. No inflammatory effect of AuNP administration was noted in control animals. **Conclusion:** Our data show that aerosolized AuNPs effectively inhibited lung function alteration and airways hyper-reactivity in silicotic mice, though failed against fibrosis response. Additional studies are needed to characterize better the mechanism involved in the suppressive effect of AuNPs on lung function alterations. **Financial support**: PAPES6/FIOCRUZ, CNPq, FAPERJ, CAPES and European Community (UE FP7- 2007-2013 - n°HEALTH-F4-2011-281608).

04.081 Parenteral administration of fish oil lipid emulsion in septic patients: clinical and biochemical responses. Messias MCF, Mecatti GC, Carvalho PO USF

Introduction: Sepsis is a serious condition with high incidence and a high mortality rate. Polyunsaturated fatty acids of the n-3 family (n-3 PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) found in fish oil, are known to modulate the immune and inflammatory responses through the production of metabolites with inflammation pro-resolving activity. In spite of the known potential benefits of fish oil, there has been little work done to evaluate the effect of this pharmaco-nutrient on patients with sepsis. This prospective, controlled, randomized, clinical trial was conducted with the aim of evaluating the occurrence of alterations to the clinical and biochemical responses of patients with acute sepsis or in septic shock who received 0.2g/Kg/day of fish oil lipid emulsion for three consecutive days. **Methods**: Adult patients in intensive care units with diagnoses of sepsis were randomized to two groups, fish oil group (n=3) and control group (n=5), and blood samples were collected before the beginning of infusion (T0) and at 24 (T1) and 72 (T2) hours after the end of infusion. Biochemical parameters were performed in serum (Cobas Mira Plus®) and total lipids of plasma and erythrocytes were extracted with chloroform and methanol (2:1 v/v), converted into methyl esters of fatty acids using BF_3 methanol and analyzed by gas chromatography (Chrompack® chromatographer model CP 9001). **Results**: No differences were found in glycemia, cholesterol, triglycerides, lactate, albumin, bilirubin, hepatic enzymes, leukocytes, platelets, oxygenation levels or coagulation test results among the times or between the groups. A significant reduction in C-reactive protein was observed in the supplemented group in the period from T0 toT1 (p=0.02). There was no difference between the two groups in regard to duration of mechanical ventilation, time spent in the ITCU, mortality, rate of acquisition of new infections or the need for hemodialysis. Plasmatic proportions of EPA and DHA increased 3.5 fold and 2 fold respectively, which led to a reduction in the AGPI n-6/AGPI n-3 ratio at times T1 and T2. There was an increase of EPA in the erythrocytes of the supplemented group measured at T1 and T2. **Conclusions**: These results show that, in the conditions prevailing in the trial, fish oil emulsion can be safely be administered and appears to have a positive effect on the systemic inflammatory responses of such patients. References: CALDER, P. C. Omega-3 fatty acids and inflammatory processes. Nutrients. v. 2, p. 355, 2010. MECATTI, G. C. Effects on biochemical and clinical parameters in patients with severe sepsis and septic shock supplemented with lipid emulsion of fish oil. Bragança Paulista: USF, 2014. 71 p. Dissertation (Master's Degree in Health Sciences) - Graduate Program *Stricto Sensu* Health Sciences, São Francisco University, BragançaPaulista, 2014. Financial Support: Support Foundation of São Paulo State Research (FAPESP). Approval: The project was submitted to the Research Ethics Committee (CEP) of São Francisco University, was approved under the CAAE 08510212.7.0000.5514 and was conducted according to the Helsinki Declaration.

04.082 LPS increase the Siglec-5 expression on human neutrophils. Amaral FC[1], Lorenzini CB[1], Macauley M[2], Spiller F[1] [1]UFSC – Farmacologia, [2]The Scripps Research Institute – Cell and Molecular Biology, Immunology and Microbial Science, and Physiological Chemistry

Introduction: Neutrophils are the most abundant circulating cells of the immune system and play a key role in host defense by different cytotoxic mechanisms. However, excessive activation of neutrophils contributes to severe tissue injury during inflammatory response. Inhibitory receptors, such as the Siglec (sialic acid-binding immunoglobulintype lectins) receptors, are potential target to avoid excessive activation of these cells and tissue damage. Siglecs are a family of receptors widely expressed in immune cells and have an important role in dampen innate and adaptive immune response. Therefore, the aim of the present study was to evaluate the expression of Siglec receptors on human neutrophils stimulated with LPS and correlated this expression with neutrophil activation. **Methods:** To determine the profile of Siglecs on neutrophils, human peripheral blood was stained with antibodies against CD66b, Siglec-1, -2, -6, -5, -8, -9 and -10 and were evaluated by flow cytometry (FACS). After this, whole blood was stimulated with LPS (1.0 µg/mL) for 3 hours and the Siglec expression were evaluated by FACS using antibodies against Siglec-3, -5, -7 and -9 as well as antibodies against CD62L and CXCR2 (markers of neutrophil activation). **Results:** Our results showed that human neutrophils (CD66b+ cells) have a high expression of Siglec-5 and -9, moderate expression of Siglec-3 and -7 and do not express Siglec-1, -2, -6, -8 and -10. Treatment of neutrophils with LPS for 3 hours induced a significantly increased of the Siglec-5 expression (increment of 100%, $p=0.03$, $n=4$) and a low increased on the Siglec-3, -7 and -9 expression (increment of 60%, 20% and 20%, respectively, $n=4$). LPS stimulation also significantly decreased the expression of CD62L and CXCR2, confirming that these cells were activated by LPS. **Conclusion:** Our preliminary results suggested that LPS-stimulated whole blood induced increased of the Siglec-5 expression on neutrophils. **Financial support:** Capes, CNPq and TSRI. This study was approved by the Ethics Committee in Research with Human Beings of UFSC, n° 283/08.

04.083 Effects of binge-like ethanol exposure during adolescence on febrile response in rats. Telles TMBB[1], Oliveira BMT, Lomba LA, Leite-Avalca MCG, Correia D, Zampronio AR
UFPR- Farmacologia

Introduction: Fever is a hallmark of infection, peripheral inflammation and an important adaptative response to the presence of pathogenic agents. Ethanol exposure during different phases of life may increase the risk of infections and promote alterations in the central nervous system. Considering that adolescence is a vulnerable period to the central nervous system and that the relationship between ethanol and febrile response is not well understood the aim of the present study was to investigated if ethanol administration, in a binge-like pattern, to adolescent rats would have late effects on lipopolysaccharide (LPS) and interleukin-1β) IL-1β (induced febrile response in animals. **Methods**: Ethanol 3 g/kg (25% w/v in saline, intraperitoneally) was administered to Wistar rats on postnatal day 25 (pre-treated group with ethanol) or saline at equivalent volume (control group). On postnatal days 26, 29, 30, 33, 34, 37, and 38 animals received the same treatment. To evaluate late febrile response, the animals were divided in two groups: 1) Twelve days after binge-like exposure (postnatal day 50) animals received an additional dose of ethanol, by oral route; 2) twenty-four days after binge-like exposure (postnatal day 62) animals received an additional dose of ethanol, by oral route. Febrile response experiments were performed on the following day (postnatal day 51 or 63, respectively) when animals were treated with saline, LPS (50 or 5 µg/kg, i.p.) or IL-1β 3) pg/ 2µl, i.c.v.). All experiments were conducted at 28°1 ± C. Hematological parameters, the status of peritoneal macrophages and plasma and cerebrospinal IL-1β levels were also evaluated on postnatal day 51. The results obtained for febrile response were evaluated by two-way repeated measures analysis of variance (ANOVA) followed by Bonferroni's test for multiple comparisons. Data for cytokine levels were analyzed by one-way ANOVA followed by Bonferroni test. **Results** and conclusion: Binge-like ethanol exposure of adolescent rats significantly reduced the febrile response induced by LPS 5 µg/kg (64%), LPS 50 µg/kg (39%) or IL-1β (%59) on postnatal day 51 when compared to saline-exposed animals. This reduction was not observed when the febrile response was evaluated on postnatal day 63. Acute administration of ethanol did not change the febrile response induced by LPS. Binge-like ethanol exposure during adolescence also did not alter hematological parameters or the number or viability of peritoneal macrophages. Binge-like ethanol exposure did not alter plasma IL-1β levels but reduced (56%) the cerebrospinal fluid levels of this cytokine .The main finding in the present study supported our hypothesis that binge-like ethanol administration during adolescence reduces the febrile response. These effects were evident for days after ethanol exposure and dissipated thereafter. The reduction may involve changes in the LPS/IL-1β signaling pathway. **Financial support**: CNPq. All procedures were approved by the Institutional Ethics Committee of UFPR (protocol # 813).

04.084 Everolimus, a mTOR inhibitor, enhance irinotecan-induced experimental intestinal mucositis by activation of proinflammatory cytokine.s Carvalho LL[1], Wong DVT[2], González RH[1], Batista GLP[1], Fernandes C[1], Nobre LMS[1], Teixeira MA[1], Magalhães V[1], Silva KO[1], Almeida PRC[2], Lima-Júnior RCP[1] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Patologia e Medicina Legal

Introduction: Colorectal Cancer (CRC) is one of the most prevalent malignancies worldwide, being one of the leading causes of cancer death. Irinotecan is commonly used as first line treatment for metastatic CRC. However, one fourth of patients undergoing treatment with irinotecan shows severe intestinal mucositis as a side effect. Preliminary data from our group showed that irinotecan-injected animals present increased mRNA expression of PI3K/Akt/mTOR pathway. Therefore, we aimed to investigate the role of mTOR during irinotecan-induced intestinal mucositis in mice. **Methods:** Male C57BL/6 mice (n=6/group) were intraperitonally (i.p) administered with saline or irinotecan (45 mg/kg) for four consecutive days. Another group of animals was given everolimus (a mTOR inhibitor, 3 mg/kg) daily followed by irinotecan injection. Body weight variations, diarrhea scores, white blood cell count were assessed. After animal euthanasia, ileum samples were collected for determination of myeloperoxidase activity, histopathology and morphometric analysis and levels of cytokines (KC, IL-1beta, IFN-gamma and TGF-beta). ANOVA/Bonferroni's or Kruskal-Wallis/Dunn's tests were used for statistical analysis. $P<0.05$ was accepted. **Results:** Irinotecan significantly reduced animal body weight (87.17 ± 3.214), white blood cell count (3,210 ± 0.518), villus/crypt ratio (1.121 ± 0.055) and also increased diarrhea scores (0[0-4]), MPO activity (779.8 ± 301.2) and IFN-gamma levels (2.09 ± 0.76) when compared to saline control group (104.6 ± 1.134; 6,582 ± 0.919; 1.871 ± 0.111; 0[0-0]; 1,242 ± 190.1, 0.83 ± 0.14, respectively). Interestingly, everolimus aggravated some of these parameters (diarrhea score: 2[0-4]; MPO activity: 3,209 ± 1,015 and IFN-gamma level: 5.74 ± 0.75) in comparison with irinotecan group ($P<0.05$). **Conclusion:** We found that mTOR inhibition aggravates irinotecan-induced intestinal mucositis by increasing proinflammatory response. **Financial support**: CNPq and CAPES. This study was approved by local ethics committee (protocol number Nº 100/14).

04.085 Anti-hyperalgesic and anti-inflammatory activity of ethanolic extract obtained from *Piper glabratum* in mice. Leitão MM[1], Navarini VJ[1], Mota J[2], Kassuya CAL[1] [1]UFGD – Ciências da Saúde, [2]UEMS – Química

Introduction: *Piper glabratum*, popularly known as pariparoba or false Jaborandi, is present in tropical and subtropical regions, is widely used in folk medicine to treat wounds and bruises, suggesting potential anti-inflammatory activity. The aims of present study was to evaluate the anti-inflammatory and anti-hyperalgesic of ethanolic extract of *P. glabratum* (EEPG) in models of complete Freund's adjuvant (CFA) and carrageenan experimental inflammation. **Methods:** In CFA model, the mechanical hyperalgesia (electronic von Frey analysis), Cold (acetone test) and oedema (plethysmometer measurement) was analyzed in three groups of C57BI6 mice (n=8) for 15 days after of 50 µL of oil suspension of CFA was administered in the right hind paw. The animals were treated orally with single administration of the EEPG (100 mg/kg), control group received saline (0.9 %) and a positive control dexamethasone (1 mg/kg, s.c.) daily. In pleurisy test, female *Swiss* mice (n=5) were divided in 3 groups: Naive, negative control (saline, p.o.), experimental groups (EEPG 100 and 300 mg/kg, p.o.) and positive control group (Dexamethasone 1 mg/kg, s.c.). After 1 hour from their respective treatments, a intrapleural injection of carrageenan 1 % (100 µL) was made while naive group received only sterile saline injection (100 µL). After 4h, the animals were euthanized and the thoracic cavity was washed with 1 mL of phosphate-buffered saline (PBS). The total number of leukocytes was determined by KX-21N unit of Sysmex. **Results:** Oral administration of EEPG significantly reduced the mechanical hyperalgesia induced by CFA in day 4 (P <0.001) and 5 (P <0.01) after the injection of the CFA, a decrease of 100 % of hyperalgesia induced by CFA. In persistent paw edema induced by CFA, the EEPG significantly reduced the volume on day 3 (P <0.001), 4 (P <0.05) and 5 (P <0.05) day with inhibition of 33 ± 4%, 25 ± 7% and 32 ± 4% respectively. In the cold sensitivity, EEPG significantly decrease the thermal sensitivity at 52 ± 4% (P <0.01) compared to the control group. In pleurisy induced by carrageenan, EEPG (100 mg/kg) reduced leukocyte migration with 64 ± 4% (P <0.01) inhibition compared to the control group. **Conclusion:** Based on the results obtained in this study, the EEPG has antiedematogenic activity, anti-hyperalgesic and inhibits leukocyte migration in the doses and models tested. In this case, further studies may be performed to elucidate the mechanisms of action. **Financial support:** CAPES, CNPq and FUNDECT. **Number of research approval by the Animal Research Ethical Committee:** 024/2014 - Ethics Committee for Animal Use - CEUA - Federal University of Grande Dourados – UFGD.

04.086 Aerobic training associated to low level laser contributes for the protection of the cardiovascular system in experimental monoarthritis. Zamuner LF, Silva A, Silva MP, Sanches IC, Angelis KD, Chavantes MC, Zamuner SR Uninove

Introduction: Studies suggest that local and systemic inflammation causes autonomic changes in the development of monoarthritis. The release of pro inflammatory cytokines implies damage to the cardiovascular system. Non-invasive therapies such as low level Laser terapy (LLLT) and aerobic training (AT) seem promising treatment in these conditions. However, the effects of AT associated with LLLT on the autonomic modulation in acute monoarthritis are unclear. **Aim:** To evaluate the association of non-pharmacological therapies of aerobic training and laser therapy on the inflammatory process and its influence on cardiovascular autonomic regulation on experimental model of monoarthritis. **Method:** Male Wistar rats (n=38) were divided into sedentary control (C=6); monoarthritis (MO=8); MO + LLLT (MOL=8), MO + AT (MOT=8) and MOT + LLLT (MOTL=8). MO was induced by intra-articular injection of zymosan (1 mg/50 µL saline) into the right knee joint. AT treatment was done with moderate AT treadmill for 8 weeks (5 days/week) and LLLT was applied at 660 nm (5mW power, power density of 0.1 W/cm2 power density of 2.5 J/cm2, 0.04 cm2 spot area and irradiation time 20 sec, twice a week). Functional capacity, arterial pressure (AP), heart rate (HR), variability pulse interval (VAR IP), low frequency band (LF) and morfomonucleares cells were measured. **Results:** Trained rats showed an increased in functional capacity in the maximum stress test (MO, INITIAL 9.17 ± 1.9 min and FINAL 8.19 ± 1.3 min; MOTL, INITIAL 7.8 ± 0.9 min and final 16.7 ± 1.9 min) ($p<0.0001$). AP values were, C, 115 ± 7 mmHg; MO 116 ± 19 mmHg; MOL 117 ± 4 mmHg; MOT 109 ± 11 mmHg; MOTL 118 ± 8 mmHg, indicating normotensive rats. HR values (C, 343 ± 25 bpm; MO, 386 ± 29 bpm; MOL 346 ± 30 bpm; MOT 324 ± 15 bpm; MOTL 328 ± 19 bpm) ($p<0.0382$) indicating an improvement of basal frequency. VAR IP (C, 90.9 ± 31.7 ms; MO, 39.1 ± 7.6 ms; MOL 33.1 ± 12.7 ms; MOT 96.2 ± 6.4 ms; MOTL 108.9 ± 29.0 ms) ($p=0.0009$) indicating improvement in autonomic modulation. LF% (C, 13.60 ± 1.3 %; MO, 20.8 ± 3.5 %; MOL 12.5 ± 1.1 %; MOT 16.1 ± 1.6 %; MOTL 13.6 ± 1.0 %) ($p=0.0674$) shows an increase in sympathetic activity. MN cells (C, 162.5 ± 60 cellx10^4/ml; MO, 471.7 ± 138 cellx10^4/ml; MOL, 109 ± 22 cellx10^4/ml; MOT, 165.5 ± 61 cellx10^4/ml; MOTL, 81.2 ± 30 cellx10^4/ml) ($p<0.0068$) decrease in LLLT group. **Conclusion:** Acute monoarthritis caused sympathetic activity in MO, suggesting an early autonomic dysfunction. AT associated with LLLT had beneficial effects on functional capacity, autonomic modulation, cardiovascular conditioning and reducing inflammation, conditions that contribute to systemic health. **Financial support**: UNINOVE CEUA - Np: AN009/2014

04.087 Anti-inflammatory activity of aqueous extracts of *Mikania glomerata* (Sprengel) and *Mikania laevigata* (Schultz Bip. ex. Baker). Pereira CS[1], Antunes E[2], Iwamoto R[2], Sawaya A[3], Landucci E[1] [1]FCM-Unicamp – Farmacologia, [2]FCM-Unicamp , [3]Unicamp

Introduction: The *Mikania glomerata* (MG) and *Mikania laevigata* (ML) are brazilian medicinal plants, popularly known as guaco and stands out for being prescribed since ancient times for respiratory problems, where studies related to anti-inflammatory activity justify their therapeutic use. The ML is often used and marketed indistinctly to MG (pharmacobotany specie) because they share the same habitat and present morphological similarity, chemical composition and similar medicinal uses. Among the constituents present in guaco, coumarin is found in greater quantities and stands out for contributing to the anti-inflammatory effect. In this context, this study aims to clarify the difference of potential anti-inflammatory action between these two species and coumarin through experimental models of inflammation, such as paw edema and acute pancreatitis. **Methods**: Male wistar rats (180 ± 220g) were anesthetized with isoflurane (2%) for paw edema procedure. The paw volume was measured immediately before the subplantar injection of 3 µg/paw of C 48/80 and at selected time thereafter intervals, using a hydroplethysmometer. For acute pancreatitis the animals were anesthetized with thiopental sodium (40 mg/kg, i.p.) and underwent laparotomy and incision in the abdominal area. The pancreatic duct were occluded for the infusion of 0.3 mL of sodium taurocholate (30 mg/kg), under constant flow of 60 seconds. The animals were sutured and after 4 hours sacrificed when the blood, pancreas and lung tissue were collected. Pancreatitis was studied for the presence of pancreatic edema and mpo activity. **Results**: The oral treatment with guaco extract and coumarin produced a progressive inhibition of paw edema formed. The MG reduced 56.3%, ML 68.8% and coumarin, in turn, decreased by 40% of the paw edema formed, at the last minute as compared to the control. In the acute pancreatitis was observed that animals treated with the extracts and coumarin showed no significant reduction in the pancreatic oedema, compared to their respective controls. For mpo activity, a neutrophil infiltration score, was observed that animals treated with the extracts and coumarin have not reduced the activity of this enzyme, that was significantly increased in the pancreas and lung, as measured after induction of pancreatitis. **Conclusion**: Our results demonstrate that MG, ML and coumarin promote significant anti-inflammatory effect on local edema, but at different times, and this same effect is not observed when it is a systemic edema, such as acute pancreatitis. These experiments show that there are important differences in the anti-inflammatory action of these species, however, additional studies are needed to establish the effectiveness of this mechanism. Financial Support: CAPES Approved by the Committee for Ethics in Animal Research (UNICAMP, protocol number 3509-1). **References**: BOLINA, R. C.; GARCIA, E. F.; DUARTE, M. G. R. Estudo comparativo da composição química das espécies vegetais Mikania glomerata Sprengel e Mikania laevigata Schultz Bip. ex Baker. Rev. Bras. de Farmag., vol. 19(1B), pág. 294-298, 2009. CZELUSNIAK, K.E.; BROCCO, A.; PEREIRA, D. F.; FREITAS, G. B. L. Farmacobotânica, fitoquímica e farmacologia do Guaco: revisão considerando Mikania glomerata Sprengel e Mikania laevigata Schultz Bip. ex Baker. Rev. Bras. Pl. Med, vol. 14, pág. 400, Botucatu, 2012.

04.088 Synergistic effect of IL-13 and adenosine (ADO) on lung fibroblast activation is dependent on A2A receptor. Sá YAPJ, Ciambarella BT, Silva PMR, Martins MA Fiocruz

Introduction: Fibrosis is a response associated with several chronic diseases, and is characterized by scar formation and accumulation of excess fibrous connective tissue. In the lungs, fibrosis leads to thickening of the walls and causes decrease in total pulmonary capacity. IL-13 is an important mediator of inflammation and remodeling which is hypothesized to act via adenosine production. Adenosine is a nucleoside that signals via G protein–coupled receptors (A_1, A_{2A}, A_{2B} and A_3), which was found in elevated levels in the bronchoalveolar lavage fluid from patients with diseases such as asthma and COPD. **Aims:** Fibroblasts are considered crucial cells involved in fibrogenesis. In this study we investigated the potential synergistic effect between IL-13 and adenosine on mice lung fibroblasts *in vitro*. **Methods**: Fibroblasts were obtained from lungs of normal Swiss-Webster mice by means of enzymatic dissociation with collagenase type 1. Cells were cultivated in DEMEN medium supplemented by SBF 10% until the third passage. The analyses included cellular proliferation and chemokine (MCP-1) generation, which were performed by means of ^{3}H-thymidine incorporation and ELISA, respectively. The cells were treated with adenosine receptor antagonists, at the concentrations of 10 and 30 μM), 1 h before stimulation with adenosine (ADO) (10 - 1000 μM) and rmIL-13 (5 - 40 ng/ml). All the analyses were performed 24 h post-provocation. Cells were treated with A_{2A}R antagonists ZM-241.385 and SCH 58281 (10 and 30 μM), 1 h before stimulation. Cell viability was evaluated by means of MTT assay. All experimental procedures were approved by the Committee on Use of Laboratory Animals of Oswaldo Cruz Foundation (license LW 57/14). **Results**: We noted that lung fibroblasts responded with increased proliferation rate and MCP-1 generation after stimulation with ADO and IL-13 alone, being the latter more potent than the former. Incubation of cells with adenosine receptor antagonists revealed that only ZM-241,385 and SCH 58281(A_{2A}R) prevented proliferation and MCP-1 production triggered by ADO. Interestingly, the co-stimulation with IL13 and ADO led to a synergic effect on fibroblast proliferation and activation, a response sensitive to treatment with A_{2A}R antagonists. None of the compounds tested showed any cytotoxicity. **Conclusions**: Our data show that IL-13 and ADO have the ability to induce lung fibroblast proliferation and activation, and that the combination of both mediators produce an exacerbation of fibroblast responses. Treatment with A_{2A} receptor antagonists attenuated stimulation by IL-13 and ADO, either alone or in combination. This indicates that this receptor seems to be accounted for by activation of lung fibroblasts and that this mechanism may represent an important therapeutic target in the case of fibrotic diseases. **Financial support:** PAPES6/FIOCRUZ, CNPq, FAPERJ, CAPES, Brazil.

04.089 Leukotriene B4 (LTB4) induces maturation and antigen-presentation function of Mice Bone-marrow Derived Dendritic Cells (BM-DCs). Pires-Lapa MA, Koga MM, Filgueiras LR, Jancar S ICB-USP – Imunologia

Introduction: LTB_4 is a lipid mediator that potentiates the innate immunity by enhancing the expression of the adaptor molecule MyD88 in macrophages (Wang, J Immunol, 5:2349, 2014). LTB_4 acts through a high-affinity G-protein-coupled receptor, the BLT-1, to induce pro-inflammatory cytokines generation and potentiate phagocytosis, microbial killing and leukocyte infiltration. While the effects of LTB_4 were well characterized in macrophages and neutrophils, in dendritic cell (DC), much less is known. In the present study, we investigated the effect of LTB_4 on DCs maturation and antigen-presentation function. **Methods**: Murine (BALB/c) BM-DCs were obtained after 6-day culture with GM-CSF. Cells were treated with LTB_4 (1, 10 or 100 nM) for 24 hours. DC markers (CD80, CD86, and MHC II) were evaluated by flow cytometry and expressed as fluorescence intensity (MFI) of $CD11c^+$ cells. For antigen-presentation assay, BM-DCs were pulsed with ovalbumin (100 µg/mL) and after 24 hours were extensively washed and co-cultured with CFSE-labeled splenocytes from BALB/c D011.10 mice (transgenic for ovalbumin-specific TCR). After 48h, the cell proliferation was determined by flow cytometry analysis. The co-cultured BM-DCs+DO11.10 splenocytes were isolated after 48 hrs and total RNA isolated with Trizol reagent, and cDNA was generated from total RNA. Real-time PCR was performed using SYBR Green and specific primers for Tbet and Gata3. Relative gene expression was calculated by the $2^{-\Delta\Delta CT}$ method. Data are shown in fold change expression of the target gene relative to internal control gene (HPRT). **Results:** BM-DCs were characterized as $CD11c^+/MHCII^+$ cells. Treatment of immature (iDCs) with LTB_4 increased the expression of the co-stimulatory marker CD86. The modulation observed in co-stimulatory molecule was reflected in DCs ability to present antigen. In the co-cultures of OVA-loaded BM-DCs with DO11.10 splenocytes, the antigen-specific proliferation of $CD4^+$ splenocytes was significantly increased when the mDCs have been pre-treated with LTB_4 (10 nM). Among the proliferating lymphocytes, treatment with LTB_4 expanded the lymphocyte population expressing the Th2 marker Gata3 but did not expand the population expressing the Th1 marker, Tbet. **Conclusion**: These results indicate that LTB_4 could act as a maturation stimulus for murine BM-DCs and boost their antigen-presenting function. Apparently, this effect of LTB^4 was selective for the Th2 lymphocytes. These results highlight a new axis in which LTB_4, besides its well-established role in inflammation and innate immunity, could also boost the adaptive immune response through its effect on DCs.

04.090 Effect of Nitroxyl donor on septic arthritis following *Staphylococcus aureus* **infection in mice.** Staurengo-Ferrari L[1], Miyazawa R[1], Mizokami SS[1], Domiciano TP[1], Pinho-Ribeiro FA[1], Fattori V[1], Pelayo JS[2], Casagrande R[3], Miranda KM[4], Verri Junior WA[1] [1]UEL – Ciências Patólogicas, [2]UEL – Microbiologia, [3]UEL – Ciências Farmacêuticas, [4]University of Arizona – Chemistry and Biochemistry

Introduction: Septic arthritis is a severe and rapidly debilitating disease associated with severe joint pain, inflammation, and oxidative stress. Nitroxyl (HNO), the redox sibling of NO•, has recently attracted interest as a therapeutic approach for cardiovascular disorders, cancer, alcoholism and pain. However, it remains to be determined whether HNO also serves as a bactericidal molecule to treat infectious diseases. Thus, the aim was to investigate the effect of HNO donor, Angeli's salt (AS) in the outcome of chronic *Staphylococcus aureus (S.aureus)*-induced septic arthritis in mice. **Methods:** To assess the effect of AS on mechanical hyperalgesia, edema, and clinical severity, mice were treated daily with AS (3 mg/kg, 150 µl) or vehicle (NaOH 10 mM plus saline, 150 ul) by subcutaneous (sc) route 1 hour after intraarticular (ia) injection with *S. aureus* (10^7 CFU/10 µl), and they were evaluated every other day until the 27^{th} day after inoculation. In another sets of experiments, mice were daily treated with AS (3 mg/kg, sc, 150 µl) or vehicle (NaOH 10 mM plus saline) 1 hour after the ia injection of *S. aureus* (10^7 CFU/10 µl) and at days 7, 14, 21, and 28 days after the stimulus injection, the knee joint samples were collected and processed to assess others parameters of inflammatory response (cytokine production, NF-kB activation, and oxidative stress) and the cartilage damage and bone destruction (proteoglycan content, histopathological analysis, and osteoclastogenesis). Lastly, the minimal bactericidal concentration (MBC) of AS on *S. aureus in vitro* and CFU number in synovial tissue were determined. Statistical differences were considered to be significant at $p<0.05$ analyzed by one-way ANOVA and Tukey's test. **Results:** Daily treatment with AS significantly inhibited the mechanical hyperalgesia and the inflammatory responses (edema, leukocyte migration, cytokines release and NF-κB activation, and oxidative stress) resulting in preventing disease severity (clinical score, proteoglycan level, osteoclastogenesis and histopathological changes). In addition, AS directly inhibited the growth of *S.aureus in vitro* and the CFU number in synovial tissue. **Conclusion:** Our results suggest for the first time the therapeutic potential of AS in a model of septic arthritis by mechanisms involving microbicidal effects, anti-inflammatory actions, and reduction of disease severity. **Financial support:** Decit/SCTIE/MS intermediated by CNPq and support of SETI/Fundação Araucária and CNPq. **Approval by the Animal Research Ethical Committee of Universidade Estadual de Londrina:** Process number 33358.2010.36

04.091 The role of GILZ in macrophage reprogramming, Vago JP[1], Jones S, Sugimoto MA, Lima KM, Lang T, James H, Morand E, Teixeira MM, Sousa LP – UFMG

Macrophages recognize an array of stimuli from endogenous and exogenous sources and respond with remarkable phenotypic plasticity. The M1/M2 nomenclature provides a basic classification system to distinguish pro-inflammatory macrophages from anti-inflammatory/wound-healing macrophages. Macrophages are prone to removal apoptotic cells (efferocytosis) a process that skew them to M2 and Mres phenotypes and is primordial to the resolution of inflammation program. Glucocorticoid(GC)-induced leucine zipper (GILZ) is a GC-regulated protein that mediates several GC functions including apoptosis and anti-inflammatory/pro-resolution activities. In a previous work we showed that GILZ is expressed in M2 and Mres macrophages suggesting that GILZ may be a key role in macrophage-induced resolution activities. In this study, we investigated the effects of GILZ in macrophage reprogramming in vitro and in vivo settings. Bone marrow from C57BL6 or GILZ KO mice were isolated and differentiated to bone marrow-derived macrophages (BMDMs) for 7 days. After that, the cells were stimulated with LPS (10ng/ml) + IFN (10ng/ml) or IL4 (10ng/ml) to induce M1 and M2 phenotype, respectively. To induce GILZ overexpression, the BMDMs were treated with TAT-GILZ (a GILZ fusion protein - 0,2ug/ml) 2h prior stimulus. BALB/c mice were challenged by i.pl. (intrapleural) injection of LPS (250ηg/cavity) or PBS. The peptide TAT-GILZ was administered systemically (0,2 mg/kg, i.p.). Cells in the pleural cavity were harvested 24h after LPS challenge and processed for total and differential leukocyte count, flow cytometry and qPCR analysis. After stimulus, the markers of M1 (CD80, CD86, MHCII and INOs) and M2 (CD206, FIZZ-1, arginase-1) were similarly increased in BMDMs from WT GILZ KO mice. However, the overexpression of GILZ using TAT-GILZ peptide decreased the expression of M1 markers *in vitro* and *in vivo* settings. In addition, TAT-GILZ treatment resolved LPS-induced inflammation associated with efferocytosis of apoptotic neutrophils, an important mechanism mediated by M2 macrophages. Collectively, these results show that GILZ could affects macrophage polarization and efferocytosis, two key steps in resolution of inflammation. **Financial Support:** CNPq, PRPq-UFMG, FAPEMIG and CAPES. **Research approval Animal Ethical Committee:** (CETEA/UFMG Protocol number 15/2011).

04.092 Annexin A1 depletion improves mice fertility and distorces sex ratio. Hebeda CB[1], Machado ID[1], Reif I[1], Bevilacqua E[2], Perretti M[3], Farsky SHP[1] [1]FCF-USP – Análises Clínicas e Toxicológicas, [2]ICB-USP – Biologia Celular e do Desenvolvimento, [3]Queen Mary University of London – The William Harvey Research Institute

Introduction: Pregnancy is controlled by distinct inflammatory responses. Although the role of the anti-inflammatory molecule annexin A1 (ANXA1) in different models of inflammation has been fully investigated, its involvement on reproduction and pregnancy has not been explored yet. Therefore, here, the participation of ANXA1 on reproduction and on sex ratio has been investigated. **Methods:** Wild type (WT) and ANXA1-deficient (ANXA1$^{-/-}$) Balb/c mice were matting and the number of pups by litter was determined by visualization of anatomical distance of genital organs. Spermogram, cytokines and ANXA2 and ANXA5 were determined on sperm or seminal fluid of WT and ANXA1$^{-/-}$ mice by computer assisted sperm analyses (CASA), cytometric bead array (CBA) and ELISA, respectively. X and Y chromosome on sperm was determined by PCR. Mice were matting and pregnancy was considered after vaginal plug visualization, designated as day 0,5 of pregnancy [0,5 day after coitus (DAC)]. Leukocyte profile, levels of cytokines, ANXA2, ANXA5 in the uterine fluid were measured 1,5 DAC by flow cytometry, CBA and ELISA, respectively. Blastocyst number was determined 3,5 DAC using Stereo Discovery V8 magnifying glass and implantation points in the uterine horn were identified 5,5 DAC. Levels of estradiol and progesterone were quantified on plasma samples obtained 1,5 DAC by ELISA. Estral cycle of mice was monitored by vaginal citology. **Results:** ANXA1$^{-/-}$ mice presented higher pregnancy success (42%), and generated more female pups by litter (28%) than WT mice, with no alterations on estral cycle. Sperm from ANXA1$^{-/-}$ mice did not present any functional alterations. Reduced levels of IL-6 (71%), TNF-□ (67%) and IL-10 (91%), enhanced levels of ANXA5 (87%) and no alterations on levels of IL-1□, MCP-1, INF-□, PGE2 were detected on seminal fluid of ANXA1$^{-/-}$ mice. Interestingly, expression of X and Y chromosomes were similar in sperm of WT and ANXA1$^{-/-}$ mice. Altered inflammatory profile was detected in the uterus of ANXA1$^{-/-}$ mice, as increased number of neutrophils (75%), augmented IL-6 (3.462%), MCP-1 (359%) and TNF-□ (378%) levels and reduced IL-10 (50%) and ANXA2 (82%) levels were found in the uterine fluid. No alterations were identified on levels of INF-□, LIF, GM-CSF and estradiol. ANXA1$^{-/-}$ mice also presented increased plasmatic progesterone levels (86%) in comparison to WT mice. Additionally, high number of blastocyst (130%) and implantation sites (207%) were found in the uterus of ANXA1$^{-/-}$ mice. **Conclusion:** We here demonstrated that deletion of ANXA1 increases the fertility and distorces sex ratio, which seems to be dependent on modifications on female parameters of reproduction, such as blastocyst implantation and inflammatory profile in the uterus. As 70% of female infertility is related to alterations on blastocyst attachment to the uterine epithelium (Norwitz *et al*. N. Engl. J. Med. 345, 1400–1408; 2001), the comprehension of ANXA1 role in the reproductive system may be an important therapeutic target to improve fertility. **Financial support:** FAPESP 2014/07328-4, CAPES. Research approval by the Animal Research Ethical Committee process number 541.

04.093 Lidocaine differentially affects acute and late phases of experimental silicosis in mice. Ferreira TPT[1], Mariano LL[1], Ciambarella BT[1], Filho JCA[2], Hogaboam CM[3], Martins MA[1], Silva PMR[1] [1]Fiocruz – Inflamação, [2]FM-USP – Farmacologia, [3]Cedars Sinai Medical Center

Introduction: Silicosis is an occupational lung inflammatory disease caused by silica particle inhalation, being characterized by inflammation and fibrosis - there is no treatment available until now. Lidocaine is a local anesthetic showing anti-inflammatory activity already demonstrated in allergic processes. In this study we investigated the effect of lidocaine treatment on a model of murine experimental silicosis. **Methods:** Swiss-Webster mice were instilled intranasally with silica particles (10 mg/50 µL) and the analyses were performed at different time points after challenge. Lidocaine was administered by aerosol 1 - 2% during seven consecutive days, starting 6 hours (initial phase) or 21 days (late phase) after silica instillation. All experimental procedures were performed in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (LW-57/14). **Results:** A temporal correlation was noted concerning morphological alterations including inflammatory infiltrate F4/80 positive cells (macrophages), production of cytokines and chemokines (MIP-2, KC, TNF-α e IFN-γ) and a progressive fibrosis and granuloma formation during the course of the disease. We showed that silicotic mice exhibited decreased lung function (increased airways resistance and lung elastance) and hyper-reactivity after methacholine stimulation. Treatment with lidocaine, at the initial phase, inhibited lung function alterations, macrophage infiltration, cytokine production and fibrosis, both at 7 and 28 days after silica instillation. In contrast, lidocaine administration at the late phase (21 days) failed to modify or reverse all the parameters analyzed. Additionally, we demonstrated that lidocaine inhibited TNF-α production by alveolar macrophages (AMJ2C11 lineage) stimulated with silica *in vitro*. Moreover, using bone-marrow derived macrophages differentiated *in vitro* to M1 phenotype (pro-inflammatory) and M2 phenotype (anti-inflammatory), we noted that only the M1 macrophages were sensitive to lidocaine as attested by reduced levels of cytokine release (TNF-α and IL-1β). **Conclusion:** Our results show that treatment with lidocaine was shown to be effective to interfere only with silicosis installation and failed when the physiopathology was already established. This could be attributed, at least partially, to the anti-inflammatory properties of lidocaine as shown by its ability to inhibit the secretory function of pro-inflammatory macrophages (M1). **Financial support:** PAPES VII/FIOCRUZ, CNPq, FAPERJ (Brazil).

04.094 Effect of topical gold nanoparticles formulations on cutaneous inflammation in mice. Ferreira GK[1], Olivio M[1], Soley BS[1], Paula MMS[2], Cabrini DA[3], Otuki MF[3] [1]UFPR – Farmacologia, [2]UNISUL – Ciências da Saúde, [3]UFPR

Background: Gold nanoparticles (AuNPs) have been actively investigated in a wide variety of biomedical applications because of their biocompatibility, easy conjugation to biomolecules and therapeutic properties for the treatment of inflammatory and arthritis processes. Therefore, this study aims to evaluate the anti-inflammatory activity of AuNPs on acute and chronic skin inflammation induced by 12-O-tetradecanoylphorbol acetate (TPA) in mice. **Methods:** The AuNPs were synthesized with average size of 10 nm. Acute experiment: Ear edema was induced with TPA followed by topic administration of AuNPs embedded in non-ionic cream or lotion of 0.06%, 0.1% and 0.6%, dexamethasone 0.5% (positive control) and vehicles (cream or lotion), twice a day. A digital micrometer was used to measure ear thickness before and after 6 and 24 h after TPA. At the end, animals were euthanized and the myeloperoxidase enzyme (MPO) activity was determined. Chronic experiment: Chronic skin inflammation was performed by TPA applications on alternated days during 9 days. Treatment with AuNPs occurred every 12 h starting on day 5 until the end. The ear thickness was measured daily. The MPO and n-acetyl-β-D-glucosaminidase (NAG) activities were performed after the euthanasia of animals. **Results:** **Results** showed that animals treated with AuNPs in lotion, as well as the cream formulation at 0.06% did not interfered in ear edema in 6 h. However, the cream formulation at 0.1% and 0.6% caused a decreased in ear edema. In 24 h after TPA application, all formulations and doses presented a reduction in ear edema. The most efficacious formulation was the 0.1% AuNPs cream in both periods (6 and 24 h). A decrease in MPO enzyme activity was verified in the group treated with 0.1% AuNPs cream when compared with vehicle group. In the model of repeated application of TPA, the treatment with 0.1% AuNPs cream caused a significant reduction in the edema from day 5 through 9, and on MPO and NAG activities (day 9) when compared with vehicle group. As expected, all treatments with dexamethasone formulation, the reference drug used, reduced significantly the inflammation events analyzed. **Conclusions:** Our results demonstrated that AuNPs are effective anti-inflammatory option for topical treatment and promising for skin inflammatory diseases. This work was supported by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), and Fundação Araucária. The protocol and experimental design were approved by the Animal Ethics Committee, Federal University of Paraná, number 610.

04.095 The hydrogen peroxide enhances the resolution of allergic inflammation by inhibiting ERK and NFKB. Reis AC[1], Magalhães G[2], Barroso LC[3], Costa WC[1], Perez DA[1], Silva JD[1], Souza DG[4], Teixeira MM[3], Pinho V[1] [1]ICB-UFMG – Morfologia, [2]ICB-UFMG, [3]ICB-UFMG – Biochemistry and Immunology, [4]ICB-UFMG – Microbiologia

Introduction: Eosinophil apoptosis is regarded as an important process for successful resolution of inflammation. A defective removal of eosinophils likely leads to chronic inflammatory diseases such as asthma. Thus, there is great interest in understanding the mechanisms responsible for the elimination of these cells from inflammatory sites. Previous studies demonstrate that H_2O_2 accelerated the resolution of airway inflammation by inducing eosinophil apoptosis. However, these mechanisms remains unclear. Therefore, this study aimed to investigate the mechanisms whereby H_2O_2 induces apoptosis in eosinophils. **Methods:** *In vitro:* human blood leukocytes were isolated and these cells were incubated with H_2O_2 for posterior analysis*. In vivo:* Allergic inflammation in wild-type and $gp91^{phox-/-}$ mice and H_2O_2 or signal transduction pathways inhibitors were given at the peak of inflammatory cell accumulation. The apoptosis of leukocyte was evaluated by morphological and biochemistry analysis. To investigate the mechanisms associated to apoptosis of leukocyte induced by H_2O_2, we assessed the lipid peroxidation, oxidative stress, change in the permeability of mitochondria using MitoTracker-CMX/ROS and the ERK (1/2)/NFκB expression by immunofluorescence. **Results:** H_2O_2 induced apoptosis in human leukocytes and promoted the resolution of inflammation in $gp91^{phox-/-}$ mice, which presents persistency of prolongated eosinophilia. This apoptosis was associated to inhibition of ERK/NFκB but not to lipid peroxidation and mitochondrial disfunction. In addition, H_2O_2 increased the macrophage efferocytic capacity a important event in the inflammation resolution. **Conclusion:** These results indicate a pro-resolving effect of H_2O_2 in allergic inflammation by inducing eosinophils apoptosis and inhibiting cell survival signaling pathways. **Financial supports:** CAPES, CNPQ e FAPEMIG. UFMG **Ethics Committee number** 218/2011**.**

04.096 The docosapentaenoic acid derivatives $PD1_{n\text{-}3DPA}$ and $RvD5_{n\text{-}3DPA}$ are novel effectors of intestinal protection. Gobbetti T[1], Dalli J[1], Colas R[1], Aursnes M[2], Vergnolle N[3], Deraison C[3], Hansen TV[2], Serhan CN[4], Perretti M[1] [1]The William Harvey Research Institute, [2]University of Oslo, [3]INSERM, [4]Harvard Medical School, Boston

Introduction: The resolution of acute inflammation is an active process orchestrated by specialized pro-resolving lipid mediators (SPM) that limit the host response within the affected tissue and promote homeostasis. The persistence of inflammatory signals, irrespectively of the etiopathogenesis, is the main feature of chronic inflammatory conditions sinflammatory bowel diseases (IBDs). A breakthrough was recently made with the description of a new biosynthetic pathway for docosapentaenoic acid (n-3 DPA) conversion to novel SPMs by both human and murine leukocytes (Dalli J, Sci Rep. 2014;4:6726). The aim of this study was to investigate presence and effect of n-3 DPA-derived SPM in intestinal inflammation. **Methods**: Targeted LC/MS/MS metabololipidomics was used to quantify lipid mediators derived from n-6/n-3 polyunsaturated fatty acids (PUFA) in human colon biopsies. Male 8-month C57Bl/6 mice (n=6) were subjected to colitis (5 days with 2.5% DSS in drinking water followed by 3 days water). Inflammation was assessed by monitoring colon shortening, wall thickness, myeloperoxidase activity and macroscopic/microscopic damage. Mesenteric intra-vital microscopy was performed to assess post-ischemic granulocyte recruitment (30' splanchnic ischemia followed by 90' reperfusion). Human neutrophil-endothelial interactions were assessed by flow chamber assay. **Results**: Using lipid mediator profiling we identified and quantified SPMs form the four major PUFA metabolomes in controls and IBD biopsies. LTB_4, PGE_2 and TxB_2 concentrations were increased in biopsies harvested from damaged areas of the IBD colon samples as compared to healthy tissues. In these biopsied we also identified SPM from the n-3 DPA metabolome including $PD1_{n\text{-}3\ DPA}$ or $RvD5_{n\text{-}3\ DPA}$. Systemic treatment of mice with $PD1_{n\text{-}3\ DPA}$ or $RvD5_{n\text{-}3\ DPA}$ (0.3 µg/mouse i.p. daily for 8 days) prevented colon length reduction and significantly reduced colon wall thickness, MPO activity and macroscopic/microscopic colon damage. Intra-vital microscopy demonstrated that treatment with $PD1_{n\text{-}3\ DPA}$ or $RvD5_{n\text{-}3\ DPA}$ (0.1 µg prior to reperfusion) decreased number of adherent and emigrated leukocytes in mesenteric ischemia-reperfusion. The relevance of these results to human was tested assessing the ability of these molecules to regulate human neutrophil-endothelial interactions under flow. Incubation of human neutrophils with of $PD1_{n3\ DPA}$ or $RvD5_{n3\ DPA}$ (from 10pM to 100nM) significantly ($P<0.01$) reduced their adhesion and transmigration onto TNF-α-activated endothelial monolayers compared with cells incubated with vehicle alone. Conclusion: In the present study we establish the production of n-3 DPA derived mediators in colon inflammation with both human and mouse tissues. We also found that $PD1_{n\text{-}3\ DPA}$ and $RvD5_{n\text{-}3\ DPA}$, are anti-inflammatory and tissue protective in setting of intestinal inflammation regulating neutrophil and endothelial cell responses to injury/inflammation. (Funded by William Harvey Research Foundation). Human peripheral blood was collected according to a protocol approved by Barts and the London Research Ethics Committee (London, United Kingdom [QMREC 2014:61]). All animal experiments were approved and performed under the guidelines of the Ethical Committee for the Use of Animals, Barts and The London School of Medicine.

04.097 A novel monocyte subset contributes to clearance of damage tissue during sterile inflammation in the liver. Dal-Secco D[1], Jenne C[1], Wang J[1], Wong C[1], Petri B[1], Kolaczkowska E[1], Ransohoff R[2], Charo I[3], Kubes P[1] [1]University of Calgary – Immunology Research Group, Snyder Institute of Infection, Immunity and Inflammation, [2]Lerner Research Institute – Neuroinflammation Research Center, Department of Neurosciences, [3]University of California – 3Gladstone Institute of Cardiovascular Disease and Cardiovascular Research Institute, Department of Medicine

Introduction: Monocytes are recruited from the blood to sites of inflammation, where they contribute to tissue injury clearance, wound healing and tissue repair. There are at least two subsets of monocytes: pro- ($CCR2^{hi}Cx3CR1^{low}$) and anti-inflammatory ($CCR2^{low}Cx3CR1^{hi}$). A previous study from our group using a murine model of focal hepatic necrosis induced by localized thermal injury has shown that within the initial 4 h post-injury, there is an increased directional recruitment of neutrophils to the focus of thermal-induced damage in the liver. However, it is presently unknown whether pro-inflammatory, as well, anti-inflammatory monocytes subsets also are recruited to the liver at later time-points after thermal-induced injury. Therefore, we have evaluated the dynamic of different sort of monocyte accumulation in the thermal-induced focal liver injury model from transgenic mice at later time points. **Methods and Results:** By using spinning disk confocal intravital microscopy, we have observed the generation of an intravascular chemokine (MCP-1) gradient directed pro-inflammatory monocyte migration through healthy tissue toward focus of sterile injury. Moreover, there are many pro-inflammatory (60-90 ± 0.3%/area of injury), as well as anti-inflammatory (30-90% ± 0.4/area of injury) monocytes inside the hepatic thermal lesion, as identified by transgenic animals (CCR2-RFP and Cx3CR1-GFP, respectively), after 12 to 72 h of sterile thermal injury. Interestingly, we have also identified after 24 to 72 h of thermal injury in transgenic CCR2-RFP/Cx3CR1-GFP mice, a novel double-positive population of monocytes (60-90% ± 0.7/area of injury) in the liver. These monocytes are expressing both chemotaxis receptors ($CCR2^{hi}/Cx3CR1^{hi}$). Furthermore, it was also demonstrated a significant reduction (1.5% ± 0.8 cells/area) of double positive monocyte migration, as well as insufficient necrotic cells elimination and collagen redeposition evaluated by multi-photon excitation microscopy in the hepatic thermal injury in CCR2 ($CCR2^{-/-}$), but not in Cx3CR1 ($Cx3CR1^{-/-}$) deficient mice. **Conclusion:** Therefore, our results, by dynamic in vivo imaging, revealed a third population of monocytes in the liver. Moreover, it was also demonstrated that chemotaxis receptor CCR2 is crucial for the double positive monocyte recruitment, which contributes to clearance of damaged tissue and collagen redeposition to facilitate proper wound-healing during hepatic sterile inflammation. **Financial support:** CAPES (Brazil), CIHR/IRSC and Alberta Innovates Health Solutions (Canada). All experiments involving animals were approved by the University of Calgary Animal Care Committee (protocol numbers MO8131 and MO7098) and were in compliance with guidelines established by the Canadian Council for Animal Care.

04.098 Evaluation of the effect of the ruthenium NO donor ([Ru(bpy)2(NO)SO3](PF6)) in gouty arthritis induced by monosodium urate crystals in mice. Rossaneis AC[1], Vendrameto CZS[2], Balbinot DTL[2], Staurengo-Ferrari L[2], Calixto-Campos JE[2], Bertozzi MM[2], Verri Junior WA[2] [1]UEL – Ciências da Saúde, [2]UEL – Patologia

Introduction: Gouty arthritis is a inflammatory arthritis caused by the accumulation of monosodium urate crystals (MSU) in joints (Dohert and Dieppe, 1982), that occurs due to overproduction and/or low excretion of uric acid (Wortmann, 2005).The patients with gouty arthritis exhibit sensitivity, swelling and painful joints. Additionally, the condition may result in loss of joint function and reducing in quality of life patients (Keith and Gilliland, 2007). The current drug therapy is expensive, not completely effective and has several side effects with potential toxicity. Thus, the development of new therapies to reduce inflammation and joint pain to promote the improvement in the quality of life of patients is extremely useful. Recent studies have demonstrated the therapeutic effect of NO donors in the control of pain and inflammation. The administration of ruthenium NO donor (Ru(bpy)2(NO)SO3](PF6)) reduced pain behavior and inflammatory parameters in different animal models of pain and inflammation (Staurengo-Ferrari et al., 2013, 2014). This study evaluated the therapeutic effect and mechanisms of action of ruthenium NO donor in gouty arthritis in mice. **Methods**: The experiments were conducted in accordance to the Ethical Committee for Animal Experimentation of the State University of Londrina. The mice were treated with subcutaneous injection (s.c.) of ruthenium NO donor (0.3, 1, 3 mg/kg) 30 min before intra-articular injection of MSU (100µg / 10µL / joint), as standardized in previous studies. The mechanical hyperalgesia and edema were assessed at 1, 3, 5, 7 and 15 hours after intra-articular MSU administration. After 15 hours, the mice were euthanized for evaluation of leukocyte infiltration in the joint. Other groups were treated with ruthenium NO donor (1 mg/kg, s.c.) 30 minutes before the intra-articular injection of MSU (100 µg/10µL/joint) and 15 hours after, the joints were collected for evaluation of cytokine production and oxidative stress. **Results**: The treatment with 1 or 3 mg/kg of ruthenium NO donor significantly reduced the mechanical hyperalgesia, edema and leukocyte recruitment at 15 hour induced by MSU injection. In addition, the treatment with 1 mg/kg of ruthenium NO donor significantly decreased the production of inflammatory cytokines and oxidative stress when compared to control. Conclusion: Our results indicate that the therapeutic effect of ruthenium NO donor in gouty arthritis induced by MSU crystals depends on inflammation and oxidative stress reduction, which favors the use as a future therapeutic alternative in the treatment of gout. **Financial support**: Capes, Fundação Araucária and CNPq. Research approval by the Animal Research Ethical Committee (n° 14600.2013.73).

04.099 Melatonin prevents weight gain induced by acute high-fat diet feeding in rats. da Silveira Cruz-Machado S, Pereira EP, Rocha VA, Fernandes PA, Markus RP IB-USP – Fisiologia

Introduction: Obesity, an inflammatory pathology induced by long-term high-fat diet (HFD) promotes chronic changes in hormonal and immune responses. Our group showed that acute inflammatory responses (1-7 days) blocks pineal function resulting in a decrease of nocturnal melatonin rise and increase of leukocyte migration (Markus et al., Int J Mol Sci, 14, 10979, 2013). At the first day of HFD, signals of an acute inflammatory response, such as microglia activation and cytokine production in the hypothalamus, as well as increased food intake are observed (Thaler et al., Diabetes, 62, 2629, 2013). Here we evaluated whether inhibition of pineal function might play a role in HFD-induced acute inflammatory response. In addition, we also evaluated whether the administration of melatonin at nighttime along with HFD impairs body weight gain. **Methods**: Adult male Wistar rats were kept in individual cages under a 12/12h light/dark cycle (lights on at 6h00, ZT0) receiving water and low-fat diet (Chow, 3.5% kcal from fat) or HFD (35% kcal from fat) ad libitum. Melatonin (45 ng/mL) or vehicle (0.05% ethanol) was administered in the nocturnal drinking water when indicated. Body weight and food intake were assessed daily along 7 days. Animals were euthanized at the middle of the night (ZT18) at night1 and 7 under dim red light. Plasma levels of corticosterone, insulin, leptin and melatonin were detected by Milliplex magnetic bead panels. Gene transcription was evaluated by qPCR. Data are presented as mean ± S.E.M. **Results**: Animals fed on HFD presented increased body weight gain at the day 7 when compared to low fat diet (HFD: 31.3 ± 8.0 vs Chow: 23.2 ± 1.9 g, N=5, $P<0.05$). Administration of melatonin at nighttime during 7 consecutive days prevents the increase in body weight induced by HFD when compared to rats receiving vehicle (Vehicle: 31.7 ± 2.8 vs Melatonin: 21.0 ± 1.9 g, N=5) despite maintenance of the higher caloric intake in HFD. These data strongly suggest that pineal melatonin production should be reduced at the first night of HFD intake. Indeed, plasma melatonin level at ZT18 was significantly reduced at night 1 of HFD feeding compared to chow (103.9 ± 3.7 vs 70.5 ± 6.3 pg/mL, N=3, $P<0.05$) and was still kept suppressed at night 7 (95.7 ± 3.9 vs 72.2 ± 5.6 pg/mL, N=4, $P<0.05$). The other hormones evaluated were not changed either at 1 or 7 days. Reinforcing HFD as inductor of acute inflammation, a two–fold reduction of the expression of the gene that converts 5-HT into N-acetylserotonin (arylalkylamine N-acetyltransferase) was observed in the rat pineal glands of HFD-fed rats. The expression of the enzyme that converts N-acetylserotonin into melatonin was not changed. **Conclusion**: The present study clearly shows that suppression of nocturnal melatonin production by the pineal gland is a pivotal pathophysiological change for induction of weight gain in HFD rats. In addition, the other hormones relevant to inflammatory and metabolic syndromes were not changed in the time evaluated. Here we open mechanistic-based perspective for evaluating melatonergic agonists as drugs for obesity therapy. **Financial Support**: FAPESP 2013/13691-1 and 2015/04557-5, CAPES and CNPq. Procedures were approved by IB-USP Ethical Committee (CEUA license number 194/2014).

04.100 Irinotecan-induced steatohepatitis: protective effect of probiotics. Melo AT[1], Aragão KS[1], Wong DVT[2], Freitas JA[1], Mourao LTC[1], Pereira VBM[1], Carvalho LL[1], Silva CMS[1], Almeida PRC[2], Lima-Júnior RCP[1] [1]UFC – Physiology and Pharmacology, [2]UFC – Pathology and Forensic Medicine

Introduction: Nonalcoholic steatohepatitis (NASH) is a limiting side-effect related to irinotecan, a drug used for colorectal cancer treatment. We have previously shown that the development of NASH is associated with bacterial translocation from the intestine to liver. Here, we speculate whether modulation of gut microbiota with probiotics would be a useful approach in preventing NASH, like several liver inflammatory diseases. **Methods:** C57BL / 6 mice (25-30g) were divided into experimental groups (n = 8-10) and injected thrice a week every other day with saline (5 ml/kg, ip) or irinotecan (50 mg/kg, ip) alone or in combination with daily injection of probiotics suspension (Simfort®, which contains *Lactobacillus acidophilus, Lactobacillus casei, Lactococcus lactis, Bifidobacterium bifidum e Bifidobacteirium lactis;* $1x10^7$ CFU/mL, p.o.). Body mass and diarrhea scores were assessed weekly. At week 5, blood samples were collected for total leukocyte count and following euthanasia liver and intestinal samples were obtained for assessment of histopathology, inflammatory parameters (neutrophil accumulation in the liver) and measurement of lipid content. $P<0.05$ was accepted. **Results:** Irinotecan caused a pronounced leukopenia, increased diarrhea severity and intestinal damage [3206 ± 398.6; 1 (0-4); 10 (7-12)] vs. saline group [(6400 ± 902.4; 0 (0-0); 4 (3-5)], which were significantly prevented by probiotics [6113 ± 683.5; 0 (0-1); 5 (3-8)]. In addition, liver histopathological scores [4.5 (2-6)] and the number of inflammatory foci [7.6 ± 2.0] were increased in irinotecan group when compared to saline-injected animals [3 (2-3); 0.6 ± 0.4]. These parameters were prevented in the animals treated with probiotics [3 (2-3); 2.7 ± 0.6]. Irinotecan caused a marked increase in liver lipids content 49.4 ± 2.5 *vs.* saline group 34.6 ± 1.9. This behavior was prevented by probiotics treatment 38.02 ± 4.3. Irinotecan-associated loss of body mass was unaffected by probiotics treatment ($P>0.05$). **Conclusion:** Probiotics prevented irinotecan-related NASH probably by the control of intestinal mucositis. The mechanisms involve merits to be investigated. **Financial support**: CAPES, CNPq and FUNCAP. **Animal research ethics committee:** 21/12. **Keywords:** Steatohepatitis. Irinotecan. Intestinal microbiota. Probiotics.

04.101 Suppression by the dominant-negative inhibitor of soluble TNF XPro 1595 of experimental silicosis in mice. Ciambarella BT[1], Arantes AC[1], Teixeira TPT[1], Szymkowski DE[2], Martins MA[1], Silva PMR[1] [1]Fiocruz – Inflammation, [2]Xencor

Introduction: Tumor necrosis factor (TNF) is a multifunctional cytokine known to regulate inflammation, which is presented on the cell surface as transmembrane TNF (tmTNF), acting to promote juxtacrine signaling, and soluble (solTNF), acting in a paracrine fashion. TNF has been shown to play an important role in fibrotic processesses. A novel class of anti-TNF biologics (DN-TNFs), which selective inhibits the soluble form of TNF has been previously described (Zalevsky J, J. Immunol, 179, 1872, 2007). Thus, this study was undertaken to investigate the effect of the XPro 1595 on the experimental model of silicosis in mice. The monoclonal antibody against TNF infliximab was used for comparison. **Methods:** Male Swiss-Webster mice were intranasally instilled with 10 mg/50uL of silica particles, and then treated therapeutically with XPro 1595 (1.25 and 10 mg/kg, i.p.) and infliximab (1.25 mg/kg) on days 7, 14 and 21 post-silica. The analyses were performed 24 h after the last dose and included the following parameters: i) lung function and airways hyper-reactivity to methacholine (invasive plethysmography -Finepointe Buxco System), ii) morphology and morphometry (H&E and picrus sirius), iii) collagen deposition (Sircol method) and iv) chemokine/cytokine generation (ELISA). All experimental procedures were performed in accordance with guidelines of the Committee on Use of Laboratory Animals of FIOCRUZ (LW-57/14). **Results:** We showed that stimulation with intranasal silica led to an increase in the basal levels of lung resistance and elastance as well as airways hyper-reactivity to aerosolized methacholine. A marked tissue leukocyte infiltration accompanied by fibrosis (collagen deposition and granuloma formation) was also noted in the lungs silicotic mice. Increased levels of chemokines/cytokines (MIP-1□, MIP-2, IL-1□□□TGF-β, KC and MCP-1) were also noted. Therapeutic administration of XPro 1595 and infliximab significantly inhibited the exacerbation of increase resistance and elastance as well as fibrosis, including increase in collagen deposition and granuloma formation in silica-stimulated mice. Chemokine and cytokine generation was also sentitive to XPro 1595 and infliximab. **Conclusion:** Altogether our findings show that treatment with XPro 1595 and infliximab effectively inhibited some critical features of silicosis, including alteration of lung function and fibrogenic response, reinforcing the idea that TNF is implicated in this disease. They also indicate that inflammation in mouse silicosis seems to be primarily driven by sol TNF. **Financial support:** FIOCRUZ/CNPq/FAPERJ.

04.102 Transcranial Direct Current Stimulation (tDCS) modulates inflammatory process induced by Freund's adjuvant. Gamaro GD[1], Medeiros LF[2], Silva SP[1], Crespo PC[1], Sanches PRS[2], Couto CA[3], Freitas JS[2], Souza A[4], Martins OG[1], Torres ILS[2], Souza ICC[1] [1]UFPel, [2]UFRGS, [3]USP, [4]Unilasalle

Introduction: Chronic pain syndromes are associated with cognitive physiological changes, such as, chronic stress, insomnia, anxiety and irritability manifestations. And, the pain process can promote restrictions in the professional and social[1] activities. The imbalance of the nociceptive system triggers the release of inflammatory mediators and modulators. For example, brain-derived neurotrophic factor (BDNF) and pro- and anti-inflammatory cytokines (e.g. interleukins - IL6 and IL10, respectively) can participate in the mechanism of chronic inflammatory[2] pain. Studies show that non-invasive treatments, which act on the central nervous system trough electrical stimulation, are effective in treating chronic pain, such as Transcranial Direct Current Stimulation (tDCS). Therefore, the aim of this study was evaluate the effect of tDCS on morphological and biochemical parameters in rats subjected to a chronic inflammatory pain model [3]. **Methods**: 54 male Wistar rats were divided into six groups: Control, Control + sham-tDCS, Control + tDCS, CFA, CFA + sham-tDCS, CFA + tDCS. The control group received saline injection and CFA group was subjected to chronic inflammation induced by Freund's adjuvant (CFA) in the right hind paw. After being established the inflammatory model in 7 days, the rats were treated for 8 days with tDCS (0,5mA, 20min / day). The morphological and biochemical parameters (BDNF, IL6 and IL10) in serum, hippocampus (HC) and prefrontal cortex (PFC) were evaluated at the end of treatment. The weight of the animals and swelling were evaluated in four stages: baseline, 7 days after induction of inflammation, immediately after treatment with tDCS and 8 days after the last tDCS session. The morphological aspects of edema in the paw of the animal were assessed by routine histological techniques with hematoxylin-eosin (HE) staining and biochemical testing using commercial kits analyzed by ELISA. Statistical analysis was by ANOVA followed by SNK, with significance of $P<0.05$ using SPSS software version 20.0. **Results**: We did not find any difference in the BDNF levels in HC and CPF, only in the serum levels, where both CFA +Sham- tDCS and CFA-tDCS presented a decrease in relation to control-tDCS group. No differences were found in the IL6 and IL10, both central as peripherally. The inflamed animal (CFA, CFA+ sham-tDCS and CFA+ tDCS) showed an increase in paw volume that was partially reversed immediately and 24 hours after tDCS treatment. The histological evaluation were captured in three animal paw areas, tarsal, cushion and heel, and analyzed through Motic Images Plus 2.0 program. Conclusion: the CFA group showed lymphocytic infiltration, increased vascularization, inflammation characteristics that were reduced by tDCS treatment. This study describes a potential anti-inflammatory action of tDCS, since tDCS has an effect on lymphocyte infiltration of edema and neovascularization according our present Results: **Financial support**: CNPq e UFPEL. Ethics Committee: CEEA/UFPEL #4538. 1. WANG, H. Y. et al. J Neuroscience, 30, 11044-11054, 2011. 2. VALLEJO, R. et al. Pain Practice, 3, 167-184, 2010. 3. CIOATO, S. G. et al. Brain Stimulation, 2015, 1-9, 2015.

04.103 Effects of high fat diet on alveolar bone loss induced by *Aggregatibacter actinomycetemcomitans* in mice. Zicker MC[1], Chaves IM[2], Laranjeira AO[2], Macari S[3], Saraiva AM[3], Duarte PM[4], Teixeira MM[5], Souza DG[2], Versiani AM[6], Silva TA[3], Madeira MFM[2] [1]UFMG – Medicamentos e Alimentos, [2]UFMG – Microbiologia, [3]UFMG – Patologia, [4]UNG – Odontologia, [5]UFMG – Biochemistry and Immunology, [6]UFMG – Nutrição

Periodontal disease (PD) is an infectious inflammatory disease which affects the supporting structures of the teeth. Changes in the host oral microbiota favor colonization by periodontal bacteria, triggering an inflammatory response that can lead to the destruction of periodontal tissues. Increased body fat has been considered a reservoir of inflammatory mediators, and thus may contribute to the activation of systemic inflammatory response with consequent impact on periodontal health. Some studies have shown that obese patients present higher incidence of PD than non-obese patients. This study aimed to evaluate the effect of a high fat (HF) diet in experimental PD induced by Aggregatibacter actinomycetemcomitans (Aa) in mice. After 4 weeks on standard or HF diet, groups of mice (n = 8) were orally infected with Aa, and diet maintained for 4 more weeks. For oral infection, mice received a direct injection of 1 x 10^9 CFU/mL of Aa strain FDC Y4 (diluted in 10 µL of PBS) into palatal gingival tissue. Immediately after, was performed an oral administration of the same inoculum diluted in 100 µL of PBS with 1.5% of carboxymethylcellulose. Negative controls received only PBS. Body weight was monitored during all experimental time. After 30 days of infection, euthanasia was performed. Thus, the following parameters were determined: alveolar bone loss, neutrophil influx (myeloperoxidase activity), cytokines (ELISA), and bacterial load (DNA-DNA hybridization). The standard diet did not induce significant weight gain and did not interfere with alveolar bone loss induced by Aa. However, HF diet was associated with spontaneous alveolar bone loss and increased adiposity. Additionally, mice fed with HF diet presented decreased IL-10 levels in periodontal tissues and less adiponectin in serum. Moreover, HF diet induced significant neutrophil influx in periodontal tissues. HF diet was not associated with increased number of Firmicutes in oral microbiota, but it was associated with increased number of Prevotella nigrescens, Fusobacterium nucleatum and Neisseria mucosa. These results indicate that HF diet may change the oral biofilm and the production of inflammatory mediators, and thus may contribute to the progression of PD. Uniterms: Periodontal disease. High fat diet. Mice. Oral microbiota. Supported by: CAPES, FAPEMIG, CNPq. CEUA/UFMG: 272/2014

04.104 SOCS2 is crucial to modulate the dendritic cells function during experimental *Trypanosoma cruzi* infection. Esper L, Gualdrón-Lopez M, Brant F, Monti-Rocha R, Pimentel PMO, Souza DG, Teixeira MM, Machado FS UFMG – Biochemistry and Immunology

Background*:* The infection by *Trypanosoma cruzi* (*T. cruzi*), which causes Chagas' disease, induces an inflammatory reaction and the efficacy of the host immune response (IR) is important to persist or eliminate the infection. SOCS2 (supressor of cytokine signaling)2 expression, an intracellular protein, is partially mediated by lipoxins (LXA_4, anti-inflammatory eicosanoid) in dendritic cells (DCs), the main antigen-presenting cell (APC). We demonstrated that SOCS2 is fundamental during *T. cruzi* infection by modulating the generation/expansion of Th1, Treg and memory cells and in the control of heart function. **Results*:*** In the present work, we investigated the role of SOCS2 in DCs function and induction/maintenance of IR during experimental *T. cruzi* infection. CD11cDTR transgenic mice (DCs depletion), wild type (WT) and SOCS2 (*knockout*/KO) were infected with Y strain of *T. cruzi*. Our results demonstrated an increased parasitemia in depleted DCs animals, emphasizing that DCs are crucial in control of *T. cruzi* infection. During innate IR, absence of SOCS2 resulted in decreased frequency of inflammatory cytokines (IL-12 and TNF-α), but not IL-10 by DCs, without change the Toll-like receptor expression (TLR2 and TLR4) and MHCII. In contrast, a decreased expression of CD80 costimulatory molecule was observed in SOCS2 deficient DCs. During adaptive IR, absence of SOCS2 in DCs resulted in increased levels of TLR2 and TLR4 expression and reduced frequency of DCs expressing MHCII. Adoptive transfer of SOCS2 deficient DCs caused increased parasitemia and changes in IR profile against *T. cruzi* infection, specifically: i) reducing the frequency of NK cells producing IFN-γ and IL-17; ii) reducing the frequency of CD8 T cells producing IFN-γ and CD4 T cells producing IL-17, despite increasing CD4 T cells producing IFN-γ; absence of SOCS2 in DCs also resulted in reduction of cells producing IL-10 such as CD4 and CD19, besides Treg cells. Our results also demonstrated that SOCS2 is important in apoptosis modulation during *T. cruzi* infection, where absence of SOCS2 leads to increased apoptosis in neutrophils during innate IR, macrophages in innate and adaptive IR and lymphocytes in adaptive IR. Our *in vitro* results demonstrated an increase in cleaved and total caspase 3 in SOCS2 deficient neutrophils. ***Conclusion*:** Together, our results demonstrated that SOCS2 is crucial in the modulation of DCs' function during generation/regulation of innate and adaptive IR during *T. cruzi* infection. **Keywords**: *Trypanosoma cruzi*, SOCS2, Dendritic Cells, Immune Response. **Supported by** CNPq and FAPEMIG. This work was approved by CEUA/UFMG access number 89/210 and 449/2015.

04.105 Down-regulation of neutrophil function by the mexiletine analogue JME-173: impact on experimental COPD. Ferrero MR[1], Coutinho D[1], Silva PMR[1], Silva ET[2], Costa JCS[2], Martins MA[1] [1]Fiocruz – Inflammation, [2]Fiocruz – Organic Synthesis of Farmanguinhos

Introduction: Chronic obstructive pulmonary disease (COPD) highly reduces the life quality of people and can be fatal. Neutrophils are well recognized as major contributors in the physiopathology of COPD. Since the current COPD therapy are highly limited, and glucocorticoids fail to reduce neutrophil inflammation, numerous research groups have investigated novel molecules as alternative for controlling neutrophil inflammation in COPD. In the present work, we assessed the anti-COPD potential of JME-173 - an analogue of the local anesthetic mexiletine, previously screened by us for anti-inflammatory activity and reduced impact on sodium channels. **Methods:** Human peripheral blood neutrophils were isolated from healthy donors who had not used medication in the week before, using the Ficoll-Hypaque separation procedure. Chemotaxis assays were performed in Neuroprobe Chemotax plates placing $2x10^5$ cells in the upper chamber and IL-8 (100 ng/ml) or fMLP (10 nM) in the lower chamber. Neutrophil viability and IL-8 production were assessed using Trypan blue and ELISA respectively. C57Bl/6 mice, treated or not with JME-173 (25 mg/kg, orally), were exposed to ambient air or cigarette smoke (CS) for 10 days, 3 cycles of 4 cigarettes per day and an inhalation time of 6 min per cigarette. Treatment starts on the sixth day of CS exposure. Neutrophils in bronchoalveolar lavage (BAL) were assessed 24 h after the last exposure to CS. All experimental procedures were performed in accordance with the guidelines of the committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (L-030/15). **Results**: We found that both IL-8- and FMLP-induced neutrophil chemotaxis were inhibited by JME-173 (IC_{50}= 60 µM), but not mexiletine, with no change in human neutrophil viability until 3 h. At 24 h, JME-173 decreased neutrophil survival (40-60%) as compared to the controls. In the presence of LPS, the viability of JME-173 treated cells decreased even more. Furthermore, JME-173 significantly inhibited LPS-induced IL-8 production by neutrophils *in vitro*. In *in vivo* settings, the interventional treatment with JME-173 almost abolished CS-induced neutrophil accumulation in the bronchoalveolar space. **Conclusion:** The findings of the current work pointed out that the interventional oral treatment with the mexiletine analogue JME-173 may down-regulate neutrophil infiltration into the lung following CS exposure. Inhibition of neutrophil survival, chemotaxis and generation of neutrophil chemokines may contribute to this therapeutic effect. Altogether these results suggest that JME-173 should be further investigated in drug development for COPD and other neutrophil-associated diseases. Financial Support: CNPq, FAPERJ, TIMER/EU and CAPES.

04.106 ATP-induced melatonin synthesis by macrophages increases phagocytic activity.
Dargenio-Garcia L, Souza-Teodoro L, Takiguchi RS, Muxel SM, Markus RP, Ferreira ZS IB-USP – Fisiologia

Introduction: Melatonin (MEL), rhythmically produced by the pineal gland but also non-rhythmically by extra-pineal sources, is described to regulate the proliferation and the activity of immune competent cells (Pontes et al, J Pineal Res. 41:36, 2006; Carrilo-Vico et al, Int. J. Mol. Sci. 14:8638, 2013). It is synthesized by the enzymes arylalkylamine N-acetyltransferase (AA-NAT) and acetylserotonin methyltransferase (ASMT). During an injury, pineal MEL synthesis is inhibited, and shifts to an autocrine/paracrine production in immune cells, a concept designed Immune-pineal Axis (Markus et al, Int J Mol Sci. 14:10979, 2013). Accordingly, in macrophages, PAMPs, such as LPS, activate Aanat transcription, inducing MEL synthesis, which leads to the expression of dectin-1, increasing phagocytosis (Muxel et al, PLos One 7:e52010, 1012, Pires-Lapa et al, J Pineal Res. 55:240, 2013). Considering that purines act as DAMP, capable of eliciting proinflammatory responses in macrophages via P2X7 receptors (Cohen and Mosser, J. Leukoc. Biol. 94:913, 2013), we evaluated whether purinergic stimulation could induce MEL synthesis by macrophages. Recently we have demonstrated that ATP inhibits endocrine MEL production (Souza-Teodoro et al, J Pineal Res. 60:242, 2016) but its role in immune cells was not evaluated. **Methods**: Murine macrophage cell line RAW 264.7 and macrophages from Wistar rats peritoneal lavage were used. MEL was measured by ELISA in the medium from cells (2x106/300µL) stimulated by ATP (0.01–3mM; 3h). The cells were also pre-treated with the NFkB inhibitor (PDTC, 25µM; 30min) or with the P2X7 receptor selective antagonist (A438079; 1µM; 30min) and then incubated with ATP (1mM, 45 or 60min) for AA-NAT or phosphorylated-AANAT (P-AA-NAT, active form) immunolabeling, respectively. The primary rabbit polyclonal anti-AA-NAT or anti-P-AA-NAT antibodies (Imuny, 1:200, 18h, 4°C) were used followed by the FITC-conjugated secondary polyclonal anti-rabbit antibody (Invitrogen, 1:200, 1h, RT). Nuclei were stained with DAPI and negative controls were assayed in the absence of the primary antibodies. The phagocytosis was assessed by the incubation with the MEL receptor antagonist luzindole (0.1, 0.3 or 1µM, 30min), followed by ATP (1mM, 3h) and then with FITC-labeled zymosan (6x106 part/well, 90min). After, cells were analyzed on AMNIS FlowSight flow cytometer (488nm laser; minimum of 12,000 single-cell events). **Results**: ATP (0,01–3mM) led to MEL production in a concentration-dependent manner (EC50=0.63 mM, CI 95%, 0.38–1.06 mM; n=3-5 wells. ATP (1mM) enhances 40% the AA-NAT enzyme protein and also P-AA-NAT through a mechanism dependent on P2X7 receptor activation and NFkB translocation. ATP-induced increase in AA-NAT protein was blocked by PDTC. The P2X7 receptor activation was confirmed by the pre-treatment with the selective antagonist A438079 that totally blocked ATP-induced increase in AA-NAT and P-AA-NAT immunolabeling. Moreover, ATP-stimulated macrophages increased the 50% the phagocytosis of zymosan, an effect blocked by luzindole in a concentration-dependent manner. Conclusion: ATP induces MEL production, which up-regulates phagocytosis unraveling a novel pathway for the control of phagocytosis by purine regulation of MEL synthesis by an immune competent cell. Support: CAPES, FAPESP, CNPq. (CEA/IB 2007/2014).