04 Inflammation and Immunopharmacology

04.001 The role of leukotrienes in inflammation, **insulin pathway and macrophage polarization in muscles in Type 1 Diabetes**. Guimarães JPT[1], Filgueiras LR[1], Martins JO[2], Jancar S[1] [1]ICB-USP – Imunologia, [2]FCF-USP – Análises Clínicas e Toxicológicas

Introduction: Type 1 diabetes (T1D) is a metabolic disease associated with systemic low-grade inflammation and macrophage reprogramming. Metabolic dysfunction is related to the persistent low-grade inflammation. Our group shown this inflammation (IL-1ß and TNF-α) depends on the high systemic levels of leukotriene-B4 (LTB4) found in T1D mice which shift macrophages towards the classically activated (M1) phenotype. Although T1D can be corrected by insulin administration, over time, T1D patients also develop insulin resistance that hinders the glycemic control, which affect the patient life quality. LTB4 is also involved in insulin resistance in metabolically active tissues including muscles in T2D. In our study we investigated the involvement of leukotrienes (LTs) in insulin resistance in muscles from T1D mice. **Methods:** T1D was induced in 129 sve mice (WT) and knockout of 5-lipoxigenase, enzyme responsible for leukotrienes synthesis ($5LO^{-/-}$) by streptozootocin (60 mg/kg, i.p) [CEUA/ICB/USP nº8/2014]. Response to insulin was evaluated by Insulin Tolerance Test (ITT), insulin concentration by ELISA and phosphorylation of Akt by Western Blotting. Gene expression of expression of insulin receptor, IL6, Stat1, MCP-1, Ym1 and Arg1 was evaluated by qPCR and IL10 by ELISA. **Results:** We observed that after a single dose of insulin (NovolinR – 1UI/Kg given i.p to T1D mice), the reduction of glycemia was more pronounced in $5LO^{-/-}$ mice compared to WT. In quadriceps and gastrocnemic muscles of T1D mice, expression of pro-inflammatory IL6 was higher in WT whereas anti-inflammatory IL10 level was higher in 5 $LO^{-/-}$ mice. Moreover, we found a higher expression of M2 macrophages markers Ym1 and Arg1 in the muscles of $5LO^{-/-}$ T1D mice and higher phosphorylation of Akt. The expression of insulin receptor gene in T1D mice was higher in $5LO^{-/-}$ compared to WT indicating a possible regulation in the expression of this gene mediated by LTs. **Conclusions:** These results suggest that LTs have impact on macrophages resident in muscles in mice with T1D and have impact on insulin receptor signaling pathway. **License number of ethics committee**: CEUA/ICB/USP nº8/2014 **Financial support**: FAPESP (2013/15719-0, 2017/11540-7), CAPES and CNPq (302903/2016-0, 301617/2016-3)

04.002 High glucose modifies macrophages response under lipopolysaccharide stimulus. Ayala TS[1], Tessaro FHG[1], Januzzi GP[1], Bella LM[1], Ferreira KS[2], Martins JO[1]
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Introduction: Hyperglycemia is one of the main sources of complications in diabetic subjects contributing to a high susceptibility to infections. A hyperglycemic environment may affect macrophages responses upon stimulation. Herein we hypothesized that hyperglycemia could change the way macrophages respond to lipopolysaccharide (LPS) stimulus. **Methods:** Bone marrow-derived macrophages (BMDM) from non-diabetic (saline) and diabetic (alloxan 60 mg/kg, i.v) from male C57BL/6 mice (CEUA/FCF/USP-488). BMDM were exposed to a normal glucose (5,5 mM) (NG) and high glucose (25 and 40 mM) (HG) media and stimulated with LPS (100 ng/mL). Toll like receptor (TLR)4 expression was measured by flow cytometry, phagocytosis was verified by opsonized red blood cells from sheep, intracellular protein was checked by Western blot, hydrogen peroxide (H_2O_2) release was determined by amplex® red hydrogen peroxide assay kit and nitric oxide (NO) measurement with GRIESS reaction. **Results:** BMDM from alloxan-treated mice expressed less TLR4 on cell surface, phagocyte less opsonized red blood cells, released less NO and phosphorylated more in p46 stress-activated protein kinases (SAPK)/Jun amino-terminal kinases (JNK) and extracellular signal–regulated kinase (ERK) 42 mitogen-activated protein kinase (MAPK) compared to BMDM from normoglycemic mice. Compared to NG medium, in HG, BMDM from non-diabetics expressed less TLR4 under LPS stimulus with higher phosphorylation at phospho-Phosphoinositide 3-kinase (PI3K) p85 and released less NO and H_2O_2 with and without LPS. When diabetic BMDM were cultured in HG, they phosphorylated less in protein kinase C-delta (PKC-δ) and in p46 SAPK/JNK, but when stimulated by LPS, they phosphorylated more in p46 SAPK/JNK. **Conclusions**: High glucose (hyperglycaemic environment) seems to modify macrophages behaviour, affecting in different aspects diabetic and normal BMDM under the same LPS stimulus. **Financial support**: FAPESP (2017/11540-7), CAPES and CNPq (301617/2016-3). **License number of ethics committee**: CEUA/FCF/USP-488 **Financial support**: FAPESP (2017/11540-7), CAPES and CNPq (301617/2016-3)

04.003 Hepatic microvascular dysfunction and increased advanced glycation end products are components of non-alcoholic fatty liver disease Pereira ENGDS[1], Silvares RR[1], Flores EEI[1], Rodrigues KL[1], Tibiriça EV[2], Daliry A[1] [1]Fiocruz – Investigação Cardiovascular, [2]INC – Pesquisa e Ensino

Introduction: The excess of caloric intake characteristic of the western diets is linked to a significant increase in metabolic syndrome (MS) prevalence worldwide. Liver abnormal fat accumulation is a feature of non-alcoholic fatty liver disease (NAFLD) and strongly associated with the MS. Recent studies have suggested that advanced glycation end products (AGEs) could be involved with the progression of NAFLD. Also, experimental models of NAFLD exhibit systemic endothelial dysfunction also seen in NAFLD patients. Interestingly, most complications leading to morbidity in MS are from vascular origin, suggesting that the vasculature is a key target in MS. Thus, in the present study, we tested the hypothesis that hepatic microvascular dysfunction is a feature of early stage of NAFLD induced by the intake of high-fat diet (HFD). Moreover, we also explore the AGE-RAGE pathway as a potential mechanism in NAFLD pathogenesis. **Methods:** The experimental model of NAFLD or MS was induced in 10 male Wistar rats by 20 weeks of feeding with a high-fat diet administration. The high-fat diet consisted of a standard rat diet modified containing 30% fat, 56% carbohydrate, and 14% protein (% g). For the non-MS control (CTL, n=10), male Wistar rats received standard rat diet for 20 weeks. Rolling and adhesion of leukocytes and tissue perfusion in hepatic microcirculation were examined using in vivo microscopic and laser speckle contrast imaging (LSCI), respectively. Oxidative stress and inflammatory parameters were analysed by TBARs, catalase enzyme activity, RT-PCR and ELISA. The participation of advanced glycation end-products (AGE) was evaluated by quantification of fluorescent AGEs in liver and serum samples. RAGE gene and protein expression was assessed RT-PCR and Western-blot, respectively. Student's t test was used for statistical significance analysis between the two groups. Differences with P values of less than 0.05 was considered significant. **Results:** Wistar rats fed high-fat diet (HFD) showed increase in epididymal and abdominal fat content, systolic arterial blood pressure, fasting blood glucose levels, hepatic triglycerides and cholesterol, and impairment of glucose and insulin metabolisms. Liver histology confirmed the presence of steatosis and ultrasound analysis revealed increased liver size and parenchymal echogenicity in HFD-fed rats. HFD causes significant increases in leukocyte rolling and adhesion on hepatic microcirculation and decrease in liver microvascular blood flow. Liver tissue presented increase in oxidative stress and inflammation. At 20 weeks, there was a significantly increase in AGE content in the liver and serum of HFD-fed rats and an increase in RAGE gene expression in the liver. **Conclusion:** The increase in liver AGE levels and microcirculatory disturbances could play a role in the pathogenesis of liver injury and are key components of NAFLD. The presence and concentration of AGEs can be proposed as a biomarker for the status of liver complications. Studies targeting AGE-RAGE pathway are in course to further determine the precise participation of AGE-RAGE pathway in liver alterations in NAFLD, which can be the initial step for the development of new pharmacological formulations to further advance in the management of patients with SM/NAFLD. **License number of ethics committee**: CEUA license L-034/2016 **Financial support**: This work was supported by grants from CNPQ, FAPERJ and PAPES/FIOCRUZ.

04.004 Effects of Anethole + Ibuprofen association on the inflammatory parameters and on the liver metabolic changes caused by adjuvant-induced arthritis. Ames FQ[1], Bracht L[2], Lima EP[1], Cuman RKN[1], Bersani-Amado CA[1] [1]UEM – Farmacologia e Terapêutica, [2]UEM – Bioquímica

Introduction: A previous study showed that the association of anethole (AN), a compound of natural origin, and ibuprofen (IB), a traditional non-steroidal anti-inflammatory drug, both at low doses, was effective for reducing the acute inflammatory response[1]. However, it is well known that some treatments which exhibit therapeutic efficacy in acute inflammatory diseases do not show the same efficacy in chronic diseases such as arthritis[2]. The adjuvant-induced arthritis (AIA), a chronic inflammatory model that has an immunological character, induces several metabolic changes in the liver of rats[3]. However, to our knowledge, there are no studies that evaluated the effect of AN and the effect of IB on the liver metabolic changes induced by AIA. **Aim:** To evaluate the effect of AN+IB association on some inflammatory parameters and on the liver metabolic changes caused by AIA. **Methods:** Holtzman rats were divided into groups (n=7/group): (*i*) normal; (*ii*) AIA; (*iii,iv*) AIA treated with AN (62.5 and 250 mg/kg); (*v,vi*) AIA treated with IB (8.75 and 35 mg/kg) and (*vii*) AIA treated with AN+IB (62.5+8.75 mg/kg). Treatment by gavage once daily was initiated on the day of arthritis induction (day 0) and maintained for 21 days. Hind paw volume, appearance of secondary lesions and the number of recruited leukocytes into femorotibial joint cavity were evaluated. Groups of rats that received the different treatments were used to evaluate L-alanine hepatic metabolism. The liver was isolated, perfused and perfusion fluid samples were collected to determinate metabolite concentrations. Experimental protocol was approved by Ethics Committee (CEUA/UEM -7896220716). Data were analyzed using ANOVA - Tukey's test ($P < 0.05$). **Results:** Treatments with 250 mg/kg AN, 35 and 8.75 mg/kg IB, and 62.5+8.75 mg/kg AN+IB reduced both injected and non-injected paws volume on the 13th, 17th and 21st days after adjuvant injection. Treatments with 35 mg/kg IB and AN+IB were the most effective. 62.5 mg/kg AN did not reduce paws volume. Treatments with AN and IB in the highest doses and AN+IB delayed the appearence of secondary lesions and reduced the number of leukocytes into the joint cavity. No significant difference was found between these treatments. On the other hand, treatments with AN and IB at low doses did not change these parameters. Treatments with 250 mg/kg AN, 35 mg/kg IB and AN+IB increased L-lactate and pyruvate production, but did not alter the low rates of oxygen uptake, glucose and urea production, and the high rate of ammonia production induced by AIA. Additionally, the treatments did not cause additive alterations on the hepatic metabolism of arthritics rats. **Conclusion:** Together, the data showed that the AN+IB has an important anti-inflammatory effect on AIA model. Treatments with AN, IB and AN+IB partially normalized L-alanine hepatic metabolism in arthritics rats. **References:** [1] WISNIEWSKI-REBECCA, E.S. et al. Chem. Biol. Interac., 242: 247, 2015. [2] GRAHAM, G.G. et al. Inflammopharmacology, 21: 201, 2013. [3] FEDATTO, J. et al. Cell Biochem. Funct. 17: 271, 1999.

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04.005 Evaluation of the activity of *Arctium lappa* **in cutaneous inflammation model**.
Pawloski PL, Hayashida MR, Mizoguti NN, Ito FY, Hirota MM, Cabrini DA, Otuki MF
UFPR – Farmacologia

Introduction: Arctium lappa, popularly known as burdock or bardana, can be found in the Formulary of Herbal Medicine, formulated by the Brazilian Health Regulatory Agency (ANVISA) since 2011, with indications like anti-dipeptide, diuretic and anti-inflammatory. As indicated by muh of the popular literature, burdock has the ability to gently stimulate health and, as a consequence, to improve the appearance of the skin. Basically, it is excellent for any skin problem, from eczema, dandruff, a wound that do not heal, or an infection such as chicken pox that results in skin eruptions, pimples, and many more. However, there is no scientific evidence about A. lappa efficacy as anti-inflammatory in skin inflammation conditions. Thus, the goal of this study was to evaluate the anti-inflammatory effect of *A. lappa* root in skin inflammation in mice, under the same conditions used by the population. **Methods:** Skin inflammation was induced by the topical application of 12-O-Tetradecanoylphorbol-13-acetate (TPA) in the ears of the mice. Animals were divided into 6 groups: Naive, control (TPA), three preparations of Burdock Tea (Infusion, Decoction 3', Decoction 5'- p.o. 6 g/mL) plus TPA, and Dexamethasone (3 mg/kg - positive control) plus TPA. Treatment with *A. lappa* or dexamethasone occurred 1 h before and again 3 h after the application of TPA. The thickness of the ear was evaluated 6 and 24 h after induction of the inflammatory process. The activity of Myeloperoxidase (MPO) and the histological analysis were performed with the samples collected from the ears of the euthanized animals after 24 h. To determine statistical significance ($p<0.05$), one-way ANOVA with Bonferroni post-hoc test was used. All experiments were approved by Animal Research Ethical Committee. **Results:** Animals treated with Burdock tea (Infusion, Decoction 3', Decoction 5' presented a significant difference on ear edema at 6 h when compared to the TPA, with a maximum reduction of 26.9% ± 7.18% in the decoction 3' group. After 24 h only the group treated with decoction 3' presented reduction in edema in 69.35% ± 11,3%. Once again Burdock tea (Decoction 3') treatment was effective reducing MPO activity in 61.77% ± 15.11%, when compared to control. Histological analysis confirmed these results for Burdock tea, showing reduction of cellular infiltration. In addition, the TPA application promoted thickening of the epidermis layer when compared with the naïve group while treatment with Burdok tea inhibited this increase in 24.99%± 9.6%, 43.39% ± 8.6% and 32.97% ± 9.6%, respectively for Infusion, Decoction 3', Decoction 5'. **Conclusion:** These results indicate that the popular use of *A. lappa* as an anti-inflammatory agent for skin problems has efficacy, specially the decoction 3, and can be used as a an alternative and/or adjuvant treatment of some inflammatory skin diseases. It is essential to extend this study with the intention of expanding the evaluation of the efficacy and safety of these burdock preparations. Acknowledgment: CNPq, CAPES and INCT. **License number of ethics committee**: 1086 **Financial support**: CNPq, CAPES and INCT.

04.006 Uvaol, **a natural triterpenoid**, **inhibits LPS-induced inflammatory response in THP-1 human macrophages**. Cavalcante-Araújo PM[1], Robert S[2], Lagente V[2], Barreto E[1] [1]UFAL – Biologia Celular, [2]University of Rennes – Pharmaceutical Sciences

Introduction: Uvaol, a pentacyclic triterpenoid found in olive oil, has attracted considerable attention because of its important biological activities including antioxidant actions. However, the effects of the uvaol on inflammatory response, especially in cytokine secretion by activated macrophages not yet been addressed. Thus, we evaluated the *in vitro* anti-inflammatory activity of uvaol in lipopolysaccharide (LPS)-stimulated THP-1 human macrophages. **Methods:** Human monocyte cell line THP-1 were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM L-Glutamine, 1% penicillin/streptomycin and 0.05 nM 2-mercaptoethanol. Cells were maintained until fifth passage in a humidified incubator at 37 ºC with 5% CO_2. THP-1 cells were differentiated into macrophages with 25 nM of phorbol 12-myristate 13-acetate (PMA) for 72 h. Next, macrophages were pretreated with uvaol 1 h prior to stimulation with 1 µg/mL LPS for 24 h. Gene expression and protein secretion of inflammatory cytokines were assessed using real-time quantitative PCR (RT-qPCR) and ELISA respectively. Additionally, cell viability was measured by MTT assay. Statistical differences were considered to be significant at $p<0.05$ analyzed by one-way ANOVA and Tukey's test. **Results:** Exposure of THP-1 macrophages to LPS significantly increased the expression of mRNA levels of IL-1β (7.4 ± 0.8 -fold), IL-6 (35.5 ± 3.2 -fold) and IL-8 (10.7 ± 0.5 -fold) after 24 h. In addition, there was also a marked increase in the secretion of IL-1β (588.3 ± 14.2 pg/mL), IL-6 (153.3 ± 17.4 pg/mL) and IL-8 (838.1 ± 14.9 pg/mL). Treatment with uvaol at 0.1, 1 and 10 µM dose-dependently suppressed the levels of gene transcription for IL-1β (in 40%, 64%, and 81%), IL-6 (in 37%, 56%, and 65%) and IL-8 (in 22%, 59%, and 68%). This treatment also resulted in significant reduction in secretion of amount of IL-1β (in 22%, 40%, and 42%), IL-6 (in 23%, 53%, and 58%) and IL-8 (in 33%, 54%, and 63%). The concentrations used for uvaol had no significant influence on viability of macrophages.

Conclusion: Our findings suggest that the uvaol affect secretion of cytokines and chemokine in THP-1 cells by suppress expression these mediators at the transcriptional level. **Financial support**: CAPES and FAPEAL

04.007 trans-Cinnamic acid attenuates inflammatory response *in vivo* **and prevents neutrophil adhesion to endothelial cells** *in vitro*. Santana JR[1], Santos SL[1], Alves PR[1], Cavalcante-Araújo PM[1], Conserva LM[2], Ferro JNS[1,3], Barreto E[1] [1]UFAL – Biologia Celular, [2]UFAL – Química de Produtos Naturais, [3]UFPE - Terras Raras

Introduction: trans-Cinnamic acid (tCA) is a phenolic compound found in fruits and vegetables and has been shown to have possess antioxidant actions. However, the effects of tCA on the acute inflammatory events, especially on neutrophil activation are yet poorly understood. In present study we aimed to investigate whether tCA may inhibit acute inflammatory response and to affect neutrophil adhesivity to endothelial cells *in vitro*. **Methods:** Male Swiss mice (25-30 g, n = 6) were intraperitoneally treated with tCA (5, 10, and 100 mg/kg) or NaCl, 0.9% solution. After 1 h, models of carrageenan (Cg)-induced paw edema and LPS-induced pleurisy were performed. The paw volume was measured 4 h after the subplantar injection of 300 µg/paw of Cg using a plethysmometer, while pleurisy was performed 4 h after LPS (250 ng/cavity) stimulation. Next, the pleural cavity exudate was collected, and leukocytes number were determined. *In vitro*, neutrophils isolated from healthy volunteers by Percoll gradient were incubated with tCA or vehicle and allowed to adhere for 2 h on human EA.hy926 endothelial cells for cytoadherence assay. Adherence of neutrophils to EA.hy926 cells was determined by direct counting and expressed as Adhesion Index (AI) as described previously (Roffê, et al., J Neuroimmunol, 142: 17, 2003). Additionally, tCA effects on TNF-α-induced shape change and ROS production were evaluated using flow cytometry. Effect of tCA on neutrophil viability was determined by MTT. The results were statistically analyzed using ANOVA followed by Tukey's test. Differences were considered significant at $p<0.05$. Experimental procedures were approved by the local animal ethics committee (License no 67/2014). **Results:** The Cg injection significantly increased paw edema (to 72.0 ± 4.4 µL), phenomenon that was inhibited by treatment with 5, 10 and 100 mg/kg tCA in 32%, 40% and 41%, respectively. In pleurisy, was observed a significant increase in total leucocyte accumulation in response to LPS (from 4.9 ± 0.1 $\times 10^6$/cavity to 23.5 ± 2.3 $\times 10^6$/cavity), especially neutrophils (from 3.6 ± 0.1 $\times 10^6$/cavity to 16.3 ± 1.8 $\times 10^6$/cavity). This increase in total leukocyte and neutrophil counting in the pleural cavity was reduced, respectively, to 43% and 42% In pre-treated animals with tCA (5 mg/kg). *In vitro*, neutrophils adhesion to EA.hy926 cells was significantly increased after 2 h (AI of 0.34 ± 0.04 to 0.78 ± 0.26), phenomenon that was inhibited in 45%, 48%, and 84%, when activated neutrophils were previously treated with tCA at 0.1, 1 and 10 µM, respectively. Treatment with tCA at 1 and 10 µM reduced TNF-α-induced shape change on neutrophils in 40% and 41%, respectively. However, these same tCA-treatments did not affect cell viability or even ROS production in TNF-α-stimulated neutrophils. **Conclusion:** These results showed the anti-inflammatory potential of tCA by suppressing acute inflammatory events as edema formation and leukocyte migration. *In vitro*, tCA was able to inhibit adhesion of neutrophils to endothelial cells without affecting another important function of neutrophils. Further investigation concerning tCA mechanism of action is in progress.
License number of ethics committee: 67/2014 **Financial support**: CNPq

04.008 Electron paramagnetic resonance detection of Nitric Oxide produced in different tissues during fever and antipyresis. Gomes BRB[1], Guimarães NC[1], Sousa GLS[2], Alves DS[2], Sousa MV[1], Souza PEN[3], Veiga-Souza FH[2] [1]UnB – Bioquímica e Química de Proteínas, [2]UnB-Ceilândia, [3]UnB – Física

Introduction: Fever is a regulated increase in body temperature and a component of the acute phase response, triggered mainly after the invasion of pathogens in the body. Besides its protective role, fever may also cause harmful effects as a result of increased metabolic rate and oxygen consumption. Thus, the balance between damages and benefits should be considered when deciding whether or not to treat a patient with antipyretics. The nitric oxide ($NO^{\bullet}$) is a molecule abundant in the body that acts as an important signaling molecule in several biological processes, such as neurotransmission, smooth muscle relaxation, blood pressure regulation, defense mechanisms, and may also be related to the signaling process during fever. **Methods:** Male Wistar rats received oral pre-treatment with dipyrone, ibuprofen, celecoxib or acetaminophen 30 min prior to intravenous injection of LPS or vehicle, which led to a reduction in febrile response in all treated animals. The animals were euthanized 5 h after the administration of LPS. For the estimation of the production of $NO^{\bullet}$, the concentration of nitrosyl hemoglobin (HbNO) in the blood was measured by Electron Paramagnetic Resonance (EPR). For the quantification of $NO^{\bullet}$ in hypothalamus, liver and brown adipose tissue, rats received injection of iron-citrate (s.c.) and DETC (i.p) 1 h prior the euthanasia. In vivo, the Fe-DETC complex is formed and traps the NO, forming an adduct with characteristic EPR signal. **Results:** The concentration of HbNO was 15-fold higher in the animals of LPS group compared to controls. Among the treated groups, only dipyrone and acetaminophen reduced the concentration of HbNO (-66% and -73%, respectively). The administration of iron-citrate and DETC inhibited the fever in rats, while it was observed an increase in the Fe-DETC-NO signal in liver and brown adipose tissue of these animals, which was prevented with the treatment with dipyrone and acetaminophen. **Conclusion:** These data suggest that there is a higher amount of trapped $NO^{\bullet}$ in peripheral tissues during fever, which may contribute for the maintenance of fever, and that reduction of the bioavailability of $NO^{\bullet}$ by dipyrone or acetaminophen may contribute to the mechanism of action of these drugs. **License number of ethics committee**: Protocol n. 100/2017 CEUA-IB/UnB **Financial support**: FAP/DF; CNPq

04.009 The role peripheral kinin receptors in atopic dermatitis. Pail PB[1], Dagnino APA[1], Neculqueo GW[2], Maccari GP[2], Campos MM[1] [1]PUCRS – Bioquímica e Farmacologia, [2]PUCRS

Introduction: Atopic dermatitis (AD) is a multifactorial inflammatory disease characterized by cellular and molecular cutaneous changes, associated with intense pruritus (Huang Curr Allergy Asthma Rep. 18: 35, 2018). Considering the role of kinin B_1(B_1R) and B_2(B_2R) receptors in itching sensation (Costa, Br J Pharmacol. 159: 888, 2010; Chen, Exp Ther Med. 12: 627, 2016), this study evaluated the relevance of B_1R and B_2R in pruritic and epidermal changes in a mouse model of AD. **Methods:** The local Animal Ethics Committee approved the experimental protocols (Protocol 15/00489). CF-1 male mice (25-30 g; 6-week-old; n = 118) were used. For AD induction, mice were previously sensitized by a single application of oxazolone (0.5%; 10 μl) in the shaved nape. Seven days after, oxazolone was reapplied to the same region, at 2- or 3-day intervals, for 16 days (days 0, 2, 4, 7, 9, 11, 14 and 16). The scratching bouts were recorded for 60 min, 30 min after the last oxazolone application, on day 16 (Tsukumo, J Pharmacol Sci.113: 255, 2010). The skin was collected for histological assessment of epidermal thickness, as an indicative of AD-associated hyperplasia. The kinin receptor expression in the skin was determined by immunohistochemistry. The selective B_1R R715 (438 nmol/kg) or B_2R HOE140 (50 nmol/kg) antagonists were administered by the intraperitoneal route (i.p.), 30 min before the assessment of pruritus. Separate experimental groups received the same doses of R715 or HOE140, given i.p., on days 8, 10, 13 and 15 of the AD induction protocol. **Results:** The acute administration of either R715 or HOE140 failed to significantly inhibit the AD-related scratching behavior. Alternatively, the repeated administration of HOE140 significantly reduced the pruritus in the AD model induced by oxazolone (53 ±8%). Of note, the chronic treatment with HOE140 markedly prevented the epidermal hyperplasia in this experimental model (86 ±10%). AD was associated with a 2-fold increase in the expression of both kinin receptors in the skin. **Conclusion:** The inhibition of kinin B_2R by HOE140 might represent a promising alternative for the management of AD. Based on the increased expression of both kinin receptors in the skin after AD induction, further experiments are in progress to assess the effects of higher doses of R175 in this experimental paradigm. **License number of ethics committee**: Protocol 15/00489 **Financial support**: CNPq, CAPES, FINEP, PUCRS.

04.010 Comparative effects of dexamethasone and a hydrogen sulfide (H_2S)-releasing derivative on atopic dermatitis in mice. Coavoy-Sánchez SA[1], Cerqueira ARA[1], Feitosa KB[1], Teixeira SA[1], Soares AG[1], Santagada V[2], Caliendo G[2], Costa SKP[1], Severino B[2], Muscará MN[1] - [1]ICB-USP – Pharmacology, [2]Università degli Studi di Napoli Federico II – Pharmacy

Introduction: Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by pruritus and eczematous skin lesions, associated with enhanced T-helper2 lymphocyte response, which results in elevated serum immunoglobulin E (IgE) concentrations. It affects 30% of children but is also highly prevalent in adults. We have previously shown that H_2S-donors reduce skin inflammation and pruritus (both histamine-dependent and independent) in mice[1,2]. Since the therapeutical potential of H_2S-donors on AD has not been studied to date, we decided to compare the effects of dexamethasone (Dexa) and a H_2S-releasing derivative (Dexa-H_2S) using a murine AD model. **Methods:** The experimental protocol was approved by the local Ethics Committee for Animal Experimentation (CEUA-ICB/USP; n° 129/2016). Female BALB/c mice (6-8_week-old) had the dorsal hair shaved and 200 μl of 0.5% 2,4-dinitrochlorobenzene (DNCB) in acetone/olive oil (3: 1) were topically applied on days 1-3. On days 15, 17, 19 and 22, the mice were topically challenged with 200 μl of 0.2% DNCB on the dorsal skin and 20 μl on the right ear. On days 19-23 after sensitization, mice were topically treated with equimolar doses (62.5, 125, 250 or 500 nmol/mice) of either Dexa or Dexa-H_2S, and 1000 nmol/mice of thiobenzamide (TBZ, the H_2S-releasing moiety). Additional groups of animals were orally treated with equimolar doses (2.5 or 7.5 μmol/kg) of either Dexa or Dexa-H_2S. **Results:** Topical DNCB induced AD-like skin lesions, scratching behavior, elevated serum IgE, ear edema, eosinophilia, augmented spleen weight and increased total number of splenocytes. Topical treatment with 62.5, 125, 250 or 500 nmol/mice Dexa-H_2S significantly reduced the skin severity score (59.2%, 37.6%, 27.9% and 28.5% respectively; $P<0.001$), scratching behavior (74.8%, 77.3%, 88.8% and 50.6% respectively; $P<0.001$), ear edema (74.2, 77.5, 98.8 and 66.4% respectively; $P<0.05$), and decreased the spleen weight, total number of splenocytes and number of eosinophils below the values observed in the animals without AD. However, TBZ did not cause any significant change of these parameters. Oral treatment with 2.5 or 7.5 μmol/kg Dexa-H_2S significantly reduced the skin severity score (24.7% and 35.2% respectively; $P<0.001$), decreased spleen weight and total number of splenocytes below the control values, and 7.5 μmol/kg dose reduced scratching behavior (43.6%; $P<0.001$). Dexa-treated animals exhibited similar responses, except for skin severity score (at topical 500 nmol/mice, Dexa-H_2S was significantly more effective than Dexa: 28.5% vs. 10.2% reduction; $P<0.05$). **Conclusions:** The presence of a H_2S-releasing moiety does not intefere with the beneficial effects of topical Dexa, and may even improve these effects at high doses. Dexa-H_2S may thus represent a potentially new therapeutic agent for treatment of AD. **Financial Support:** FAPESP, CAPES and CNPq. **References:** [1]Rodrigues et al., Pharmacol Res 2017; 115: 255. [2]Coavoy-Sánchez et al., Pharmacol Res 2016; 113(Pt A): 686. **License number of ethics committee**: CEUA-ICB/USP; n° 129/2016 **Financial support**: FAPESP, CAPES and CNPq

04.011 *Moringa oleifera* **seed oil benefits physiological and pathological wound healing**. Ventura ACSSB, de Paula T Cretella AB, Otuki MF, Cabrini DA UFPR – Farmacologia

Introduction: Wound healing occurs in order to restore skin barrier and involves a coordinated sequence of interactions between molecules and cells resulting in inflammation, re-epithelization, tissue formation and remodeling. Defects in these events occur in immunocompromised individuals, leading to improper repair and chronic wound disorders. The seeds oil of the medicinal tree *Moringa oleifera* is popularly used for skin disorders and, despite the leaves extract wound healing properties had been described, the oil healing potential has never been studied. Thus, the aim of this work was to evaluate the *Moringa oleifera* seeds oil (MOSO) influence on physiological and immunosupressed wound healing and which mechanisms may be involved. **Methods:** Mice imunosuppression was induced with dexamethasone (i.m., 1 mg/kg, daily) and confirmed by counting blood leukocytes and lymphocytes number. After 17 days, a skin excision using a 6 mm punch was performed in animals anesthetized using ketamine and xylazine (i.p., 0.03 mL). Once a day until the wound closure, each treated wound was measured, photographed and submitted to topical application of MOSO. On the second day after excision, myeloperoxidase (MPO) activity and histological cellularity were quantified. To confirm the inhibition of cellular migration activity, *in vitro* MPO activity was determined after inducing mice ear edema and inflammation with TPA topical application. When the wounds closed, hydroxiproline content, relative to collagen content, was measured. Data analysis was done using Image J software and Prism. **Results:** Immunosuppression resulted in significant reduction in the blood leukocytes (68.1 ± 4.80%) and lymphocytes (75.1 ± 3.04%) and prolonged the wound healing process to the extent of 17 days. Treatment with MOSO reduced this time for 14 days, similar to control mice not immunosupressed. When applied on healthy mice wounds, MOSO caused a closured in 11 days. The second day after excision, MOSO decreased total MPO activity (53.76 +- 11.84% and 44.32+- 9.19% for not immunosupressed and immunosupressed wounds, respectively) and tended to inhibit cellularity at the wound site. In line with this, *in vitro* MPO activity was not altered by the presence of MOSO. When the wounds closed, hydroxyproline content exhibited a tendency to be higher in both groups of animals treated with MOSO (immunosuppressed and not immunosuppressed). **Conclusion:** Topical application of MOSO may possess anti-inflammatory properties, inhibiting inflammatory cellular infiltrate and facilitating physiological and pathological immunosupressed wound healing, also acting on collagen content. However, further investigations are needed in order to clear its effects and determine the security and efficacy of MOSO for wound healing. **License number of ethics committee**: 1121 **Financial support**: CAPES, CNPq and INCT

04.012 Participation of kinin receptors in the imiquimod-induced psoriasis-like skin inflammation. Soley BS[1], Pesqueiro JB[2], Bader M[3], Otuki MF[1], Cabrini DA[1] [1]UFPR – Farmacologia, [2]Unifesp – Biofísica, [3]Max-Delbrück-Center for Molecular Medicine - Molecular Medicine

Introduction: It is known that all kinin system components are constitutively expressed on skin; furthermore, both receptors are upregulated during cutaneous disorders, such as psoriasis. We have previously shown that kinin receptors are involved in the control of the keratinocyte hyperproliferative process (Pietrovski et al. 2011). However, it is unclear how the kinin receptors modulate inflammatory parameters observed in psoriasis. **Methods:** C57bl/6 wild type (WT) and knockout (KOB1, KOB2 and KOB1B2) animals were subjected to the chronic inflammation induced by *imiquimod* (IMQ). Animals were trichomized and received IMQ topical administrations (80 mg) on the back for 6 consecutive days. On the seventh day, skin samples were collected for analysis. In addition, C57bl/6 animals were submitted to the same experimental protocol, but 1h before the IMQ administration received daily the pre-treatment with captopril (10; 30 or 100 mg/kg, p.o.). **Results:** The PASI (Psoriasis area and severity index) showed improvement in knockout animals (KOB1, KOB2 and KOB1B2) submitted to IMQ model, when compared to the WT group. In addition, knockout animals showed reduction on myeloperoxidase activity, 31.11 ± 1.80% (KOB1), 30.82 ± 1.75% (KOB2) and 51.60 ± 2.28% (KOB1B2). Similarly, the N-acetyl-BD-glucosaminidase (NAG) enzyme activity was lower in the knockout groups, equal to 31.54 ± 7.41% (KOB1), 41.11 ± 1.66% (KOB2) and 29.56 ± 6.73% (KOB1B2). Histological analyzes showed reductions of 80.16 ± 6.26% (KOB1), 98.27 ± 5.92% (KOB2) and 94.01 ± 5.22% (KOB1B2) in the number of colored nuclei. Still, KOB1, KOB2 and KOB1B2 groups showed epidermis thickness declines of 9.12 ± 2.92%, 13.63 ± 6.60% and 68.59 ± 1.60%, respectively. The PCNA analysis showed reduction in the number of immunoreactive cells in 30.95 ± 5.8% (KOB2) and 62.05 ± 2.13% (KOB1B2), while no changes were observed in KOB1 group. In addition, IMQ promotes increase in the number of immunolabelled cells for cytokeratin 14, reducing this parameter in the groups KOB1 (34.15 ± 2.95%), KOB2 (75.35 ± 1.19%) and KOB1B2 (81.69 ± 1.57%). On the other hand, captopril treatment (100 mg/Kg) worsened PASI evaluation, increased in 73.09 ± 7.82% NAG activity, epidermis thickness (31.99 ± 8.16%) and the influx of inflammatory cells (51.74 ± 3.08%), when compared to the vehicle group (saline). **Conclusions:** The kinin receptors absence improves the morphological features of IMQ-induced psoriasis, where both receptors seem to modulate inflammatory cells influx. The B1 receptors seem to be also involved in the modulation of keratinocyte proliferation, while B2 receptors seems to be more related to the differentiation process of these cells. Furthermore, the increase in bradykinin levels associated with captopril administration led to worsening of inflammatory parameters associated with psoriasis. Thus, once again there are data supporting the involvement of the kinin system in psoriasis. Further studies are carried on evaluating the behavior of kinin receptors on other signs related to the chronic inflammation of psoriasis. **Acknowledgment:** CNPq, INCT and CAPES. **Reference:** Pietrovski, et al.“B1 and B2 kinin Receptor Participation in Hyperproliferative and Inflammatory Skin Processes in Mice.” J Dermatol Sci (2011). **License number of ethics committee**: Número 1185 (CEUA/BIO - UFPR) **Financial support**: CNPq, INCT and CAPES.

04.013 Involvement of Renin-Angiotensin System in the inflammatory response of diabetic mice with induced periodontal disease. Ferreira MN[1], Ribeiro BS[1], Queiroz DP[1], Brito VGB[1], Frasnelli SCT[1], Barreto AE[1], Lara VS[2], Santos CF[3], Oliveira SHP[1]
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Introduction: Periodontal disease (PD) has a high prevalence worldwide, and it is characterized by a chronic infection leading to loss of periodontal support. Diabetes Mellitus (DM) is one of the major risk factor for periodontitis. Renin-angiotensin system (RAS) is involved in the inflammatory response and diabetic complications, and also can induce destruction of periodontal tissue.The aim of this study was to evaluate the role of renin, an important component of the cascade of the RAS system, in inflammatory response of normal and diabetic mice after experimental induction of PD. Methodos: DM was induced by streptozotocin in male Balb/c mice. PD was induced in normal and diabetic mice, treated or not with renin inhibitor Aliskiren (Alisk), by bilateral insertion of a ligature around the lower first molar and kept for 14 days. Aliskiren was daily administered, by gavage at 50 mg/Kg and starting one day before PD induction and maintained until euthanasia. Gingival tissue was collected to evaluate gene expression and immunolabeling of RAS components by real time RT-PCR and immunohistochemical(IHC) analysis. The production of CRP (C-reactive protein), CXCL2, and CCL8 was evaluated by ELISA. The protocol was approved by Institutional Animal Care and Use Committees (School of Dentistry of Araçatuba-Unesp/ Process FOA-00106-2016). **Results:** PD enhanced the expression of Agt, Ace, Agtr1, and Agtr2 in normal and diabetic mice. Agtr1 expression was higher in diabetic mice with PD and Agtr2 expression was pronouncedly higher in normal mice with PD. Ace2 and Masr was constitutively expressed in both animals. PD inhibited Ace2 in normal mice but not in diabetic mice. Masr expression was inhibited in diabetic mice with PD but not in normal micewith PD. Alisk reduced gene expression of all RAS components in both animals, except for Ace2 in normal mice. To confirm the gene expression profile, we analyzed the presence and localization of RAS components by IHC in gingival tissue. Our results demonstrated positive immunolabeling for Ace, Ace2, Agtr1, Agtr2, confirming the presence of local RAS components in all layers of epithelium, and adjacent connective tissue, however the immunolabeling profile of each target was different.PD was able to increase the immunolabeling of all analyzed targets, which had a reduction when the treatment with Alisk was performed. PD increased CRP, CXCL2, and CCL8, and Alisk inhibited CRP, CXCL2, and CCL8 production in diabetic mice with induced PD.In normal mice only CCL8 was decreased. **Conclusion:** Renin contributes to exacerbation of the inflammatory response, mainly in diabetic mice with induced PD. **Financial support and Acknowledgments:** FAPESP (Grant: #2015/03965-2) and CAPES.

04.014 Mechanism of chronic anti-inflammatory action of *Croton campetris* **essential oil and β -caryophyllene constituent evaluated by granulomatous tissue formation**. Pessoa RT[1], Oliveira-Tintino CDM[2], Silva MGL[1], Oliveira MRC[1], Silva TG[2], Firmino JG[1], Menezes IRA[3] [1]Urca – Ciências Biológicas, [2]UFPE – Antibióticos, [3]Urca – Química Biológica

Introduction: The inflammatory process is a natural reaction of the body that **Aims:** to eliminate or inactivate aggressive agents in living tissues. This study evaluates one of the mechanisms of anti-inflammatory action of *Croton campestris* essential oil (CCEO) and its major constituent β-caryophyllene in chronic inflammation induced. **Methods:** Four cotton pellets were placed in the dorsal region of mice, each pellet weighing 0.01g (anestesia: with 80mg/kg ketamine and 20 mg/kg xylazine). The treatment groups included saline 0.9%, dexamethasone 5 mg/kg, CCEO 100 mg/kg and β-caryophyllene 19.88 mg/kg (p.o.). The treatment lasted ten days, and on the tenth day the euthanasia was performed. The pellets were collected and dried for 24h at 37 °C and then were weighed. Then, a homogenate was made with dry coton pellets. For total protein dosage was added a biuret containing reagent to the homogenate. After 10 min, the sample was read by 550 nm filter spectroscopy. **Results:** the pellets mass (g) of the groups treated with dexamethasone and CCEO 100 mg/kg was significantly lower than the group treated with saline, presenting a mass reduction in 52% and 22.4% respectively. In the absorbance analysis of the homogenate, it was observed that the groups dexamethasone and CCEO 100 mg/kg, showed an absorbance reduction of 31.6% and 33.3%, respectively, when in comparison to the group control, representing a low concentration of proteins in the homogenate of the tested groups in relation to the saline control. The β-caryophyllene group did not show statistical significance, demonstrating the inefficiency of this group in reducing such proteins. Granuloma formation is characterized by transudative, exudative and proliferative phases, the proliferative phase lasts from 3 to 6 days, where there is formation of the granulomatous tissue, with increase in the synthesis of collagen, penetration and proliferation of fibroblasts and a vascularization of the tissue. **Conclusion:** The CCEO present a significant anti-inflammatory potential in chronic inflammation, indicating an action of CCEO on the proliferative phase, evidenced by the reduction in the mass of the dry pellets and a reduction in the total proteins that infiltrated the granuloma. The constituent β-caryophyllene was ineffective, indicating that the action presented by the essential oil is due to another constituent, such as 1,8-cineole. **License number of ethics committee**: This study is approval protocol by the Ethical Committee on the Use of Animals (CEUA) of Regional University of Cariri with protocol number of 232/2016.1. **Financial support**: CNPq, Capes, FUNCAP.

04.015 Vinpocetine increases interleukin 10 production and inhibits skin inflammation induced by ultraviolet b radiation. Ferraz IBG[1], Rossaneis AC[2], Pinho-Ribeiro FA[2], Medeiros DC[2], Baracat MM[1], Georgetti SR[1], Verri WA[2], Casagrande R[1], Martinez RM[1] [1]UEL – Ciências Farmacêuticas, [2]UEL – Ciências Patológicas

Introduction: Vinpocetine is a nootropic drug used to improve cognition with some anti-inflammatory and antioxidant activities reported recently (Ruiz-Miyazawa et al., 2015). Excessive skin exposure to ultraviolet B (UVB) induces skin damage per se but also triggers inflammation that boosts tissue destruction. In this study, we used a model of UVB radiation-induced skin inflammation in hairless mice to investigate whether the anti-inflammatory and antioxidant activities of vinpocetine protects the skin in this model and its mechanisms of action. **Methods:** Vinpocetine (3, 10 or 30 mg/kg) was administered via oral 1 h before and 7 h after the beginning of UVB exposure. The irradiation dose used to induce skin inflammation was 4.14 J/cm^2. Mice (5 per group) were terminally anesthetized with 5% isoflurane 12 h [for matrix metalloproteinase 9 (MMP-9) and myeloperoxidase (MPO) activities, ferric reducing (FRAP) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging abilities assays], 2 h (for superoxide anion production test) or 4 h [for levels of interleukin 10 (IL-10) test] after the UVB exposure. Afterward, the full thickness of the dorsal skins was removed and used to each test. Sodium dodecyl sulphate polyacrylamide gel electrophoresis substrate-embedded zymography was used to detect enzymes with gelatinase activity. The UVB-induced neutrophil recruitment was evaluated using the MPO colorimetric assay. The measurement of superoxide anion production was performed using the nitroblue tetrazolium assay. The ferric reducing ability was determined by FRAP assay, and the ABTS radical scavenging ability was measured by the decrease of absorbance. Furthermore, the cytokine level was determined by an enzyme-linked immunosorbent assay (Martinez et al., 2015). Data were statistically analyzed by one-way ANOVA followed by Tukey's test, $p<0.05$. The Animal Ethics Committee (CEUA process 8909.2015.89) of the Londrina State University approved all procedures of this study. **Results:** Groups of mice treated with vinpocetine demonstrated reduction of skin MMP-9 activity that was statistically significant only in the group treated with 30 mg/kg of vinpocetine. Based on these results the dose of 30 mg/kg of vinpocetine was chosen to be used in the next experiments. Treatment with vinpocetine 30 mg/kg brought MPO activity to baseline levels, inhibited superoxide anion production, and showed antioxidant potential (FRAP and ABTS assays) statistically similar to non-irradiated group. Moreover, vinpocetine treatment increased IL-10 levels in the skin after UVB exposure**.** **Conclusion:** In summary, vinpocetin treatment increased IL-10 levels in the skin after excessive UVB exposure to block the recruitment of activated neutrophils (measured by MPO activity). This effect resulted in reduced production of superoxide anion radical and depletion of antioxidant capacity of the skin, and inhibited the proteolytic activity of MMP-9 enzyme. Thus, vinpocetine may be a promising approach to reduce UVB irradiation-induced skin damages and merits further studies. **Acknowledgments:** CAPES, CNPq, Fundação Araucária and UEL. **References:** Martinez, RM, J. Nat. Prod., 78, 1647, 2015. Ruiz-Miyazawa KW, PLoS One, 10, 3, 2015. **License number of ethics committee**: 8909.2015.89 **Financial support**: CAPES, CNPq, Fundação Araucária and UEL

04.016 Telmisartan protect against periodontal disease-induced alveolar bone loss in spontaneously hypertensive rats. Brito VGB[1], Linjardi MC[1], Barreto AA[1], Patrocino MS[1], Queiroz DP[1], Lara VS[2], Santos CF[3], Oliveira SHP[1] [1]Unesp-Araçatuba – Ciências Básicas, [2]USP – Ciências Biológicas, [3]USP – Farmacologia

Introduction: Periodontal disease (PD) is an inherited or acquired disorder of the tooth surrounding tissues and alveolar bone, initiated by bacteria biofilm accumulation. The coexistence of systemic conditions, as hypertension, can exacerbate inflammatory response and enhance bone resorption. Recent studies have demonstrated that local renin angiotensin system can have a role in PD progression. **Aims:** To evaluate the effects of telmisartan (Telm, AngII receptor type 1 blocker) in the alveolar bone loss and gene expression of bone markers on mandibles of normotensive and hypertensive rats with PD. **Methods:** 10-week old male Wistar and SHR were subjected to 15 days of PD, induced by bilateral silk ligature, placed in the first inferior molars, and were concomitantly treated with Telm (10 mg/Kg). Hemimandibles were harvested for micro-computed tomography and real-time RT-PCR analysis of bone formation (Runx2, Osx, Catnb, Alp, Col1a1, Opn, Ocn, Bsp, Bmp2) and resorption/remodeling markers (Trap, RANK, RANKL, cathepsin K, MMP-2 and -9, OSCAR). Institutional Animal Care and Use Committees Approval (School of Dentistry of Araçatuba; #00686-2016). **Results:** Telm treatment had protective effect against the PD-induced bone loss, especially on SHR. PD did not altered significantly the expression of transcription factors, but Telm was able to increase Runx2 expression. Regarding to bone formation markers, PD significantly reduced Alp expression, and Telm treatment increased its expression, especially on SHR. Opg/Rankl/Rank axis had increased expression on groups with PD, and Telm treatment reduced Rankl expression only in SHR. Bone resorption markers, Trap, Mmp9, Ctsk, Oscar, Vtn, and Itga5, were elevated by PD, on both strains, and Telm treatment was able to prevent this response. **Conclusion:** Our results suggest that Telm treatment can have beneficial effect on PD progression, by preventing the PD-induced bone loss, and increased expression of bone resorption markers, probably by blocking local angiotensin II effects on periodontal tissues. **License number of ethics committee**: School of Dentistry of Araçatuba; #00686-2016 **Financial support**: FAPESP (Grant #2015/03965-2 and #2017/070-958) and CAPES.

04.017 Fluoxetine treatment does not improve skin lesions like psoriasis in mice. Hiekis A, Pawloski PL, Brandenburg MM, Rocha FG, Otuki MF, Cabrini DA UFPR – Farmacologia

Introduction: Psoriasis is a chronic inflammatory skin disease mediated by a number of known factors, such as inflammatory cytokines, as well as mechanisms not yet fully elucidated. It is a common consensus that worsening and/or triggering of psoriasis signs and symptoms are directly linked to neuropsychiatric disorders such as depression, anxiety, and post-traumatic disorders. Therefore, the prescription of antidepressants for dermatological patients is common, and the clinical outcome is effective both in the psychiatric setting and in the improvement of the clinical signs of psoriasis. Some studies show that the serotonin system is present in the skin and may in some way be negatively regulating the inflammatory process and hyperproliferation of keratinocytes. Thus, the objective of this study was to investigate the action of different preparations of Fluoxetine, an inhibitor of serotonin reuptake, in skin inflammation models in mice. **Methods:** Chronic skin inflammatory process was induced by multiple topical applications of TPA (2.5 µg/ear) in the mice ear, on alternate days for nine days. Animals were divided in groups: Naive, Control (TPA), Fluoxetine Cream 5%, Fluoxetine 10 mg/kg (p.o.) and dexamethasone 0.1 mg/ear. Treatments started on the fifth day of the trial, twice a day. Ear thickness was evaluated every day with a digital micrometer. At the end animals were euthanized and biopsies of ear tissue were collected for analyses. Psoriasis was induced by topical application of Imiquimod (IMQ) in the back of the mice, for 6 days. The animals were divided into groups: Naive, Control (IMQ), Fluoxetine Cream 5%, Fluoxetine 10 mg/kg (p.o.) and Metotrexate (Cream 1% or 1 mg/kg, p.o. - positive control). Treatments started on day 3 until the end, twice a day. The PASI of the lesions are evaluated every day. On the seventh day of the experiment, the animals were euthanized, and biopsies of back tissue were collected and submitted to analyses. The histological analysis, the activity of the Mieloperoxidase and N-acetyl-β-D-glucosaminidase were performed with the samples collected from the both experiments. To determine statistical significance ($p<0.05$), one-way and two-way ANOVA with Bonferroni post-hoc test was used. Research was approved by Animal Research Ethical Committee. **Results:** In the TPA model, the ear thickness reduced in 7,45% +- 15,06% in fluoxetine cream group and enhanced 36,28% +- 18,15% in fluoxetine orally group, when compared with the vehicle group. The MPO and NAG activity were similar to the vehicle group, without statistic difference. In the IMQ model, the results haven't difference when compared with the vehicle group. **Conclusion:** Based on the results obtained within TPA and IMQ model of psoriasis, topical or orally use of Fluoxetine does not promote improvement of skin inflammatory conditions. Although the results obtained show no efficacy of fluoxetine on skin lesions, the importance of this study may be the beginning of understanding about the importance of serotonin and, maybe serotonin reuptake inhibitors, in skin inflammatory conditions. **License number of ethics committee**: 1047 **Financial support**: CAPES, CNPq, INCT.

04.018 Topical formulation containing 15-Deoxy-$\Delta^{12,14}$-PROSTAGLANDIN J_2 Inhibits inflammation and skin oxidative stress induced by UVB irradiation. Bezerra JR[1], Martinez RM[1], Kumagai CM[1], Saito P[1], Colombo BB[1], Melo CPB[1], Baracat MM[1], Georgetti SR[1], Verri WA[2], Casagrande R[1] [1]UEL – Ciências Farmacêuticas, [2]UEL – Ciências Patológicas

Introduction: Skin exposure to UVB irradiation increased significantly over the last years due to ozone depletion and represents the main cause of many skin diseases. 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2) is an active lipid compound derived from arachidonic acid, that influences multiple signaling pathways. Studies have revealed that it has anti-inflammatory, antiangiogenic, as well as a significant anticancer effect (BIE et al., 2018). However, there is no scientific evidence on *in vivo* topically active formulation containing 15-d-PGJ_2 to prevent and/or treatment photodamage. Thus, the present study aimed to investigate the potential use of topical formulation containing 15-d-PGJ_2 to prevent and/or reduce UVB irradiation-induced skin inflammation and oxidative stress. **Methods:** Hairless mice were randomly designed to different groups with 5 mice each: non-irradiated control, irradiated control (4.14 J/cm^2), irradiated and treated with formulation without 15-d-PGJ_2, irradiated and treated with the formulation containing 15-d-PGJ_2 (30, 90 or 300 ng). Mice received topical treatment on the dorsal surface with 0.5 g of the formulation, 1 h and 5 min before and 5 min after the irradiation session. Samples of skin were collected 2 h (catalase test), 4 h [lipid hydroperoxides (LOOH) test] or 12 h after (other tests) after the end of the irradiation. The skin edema was measured as an increase in dorsal skin weight. The UVB-induced neutrophil migration was evaluated by myeloperoxidase (MPO) activity assay. Sodium dodecyl sulphate polyacrylamide gel electrophoresis substrate-embedded zymography was used to detect enzymes with gelatinase activity. The LOOH production was determined by tert-butyl hydroperoxide-initiated chemiluminescence assay. The ferric reducing ability was determined by FRAP assay, and the ABTS radical scavenging ability was measured by the decrease of absorbance. The GSH levels were determined by the 5,5'dithiobis (2 nitrobenzoic acid) (DTNB) assay (Martinez et al., 2015). The catalase activity was evaluated by measuring the decay in the concentration of hydrogen peroxide (Martinez et al., 2015). Data were statistically analyzed by one-way ANOVA followed by Tukey's test, $p<0.05$. The Animal Ethics Committee (CEUA process 1447.2015.10) of the Londrina State University approved all procedures of this study. **Results:** Topical formulation containing 15-d-PGJ_2 inhibited UVB irradiation-induced skin edema, MPO activity, MMP-9 activity, lipid peroxidation and depletion of antioxidant capacity (ferric and ABTS reducing abilities, reduced glutathione levels, and catalase activity). Formulation containing 15-d-PGJ_2 inhibited UVB irradiation-induced photodamage in a dose-dependent manner. **Conclusion:** Formulation containing 15-d-PGJ_2 protected the skin from UVB-induced inflammation and oxidative stress in mice. Thus, these data suggest 15-d-PGJ_2 containing formulation as a potential product for the treatment of skin photo-damage and, potentially, for other inflammatory and oxidative skin diseases in which excessive **Acknowledgments:** CAPES, CNPq, Fundação Araucária and UEL. **References:** BIE, Q, Am. J. Transl. Res., 10, 648, 2018. Martinez, RM, J. Nat. Prod., 78, 1647, 2015 **License number of ethics committee**: 1447.2015.10 **Financial support**: CAPES, CNPq, Fundação Araucária and UEL

04.020 The Role of (De)nitrosylation in Inflammatory Parameters of Pneumosepsis Pathogenesis Oliveira FRMB[1], Rosales TO[1], Mattos JEL[1], Assreuy J[2] [1]UFSC, [2]UFSC – Farmacologia

Introduction: Protein S-nitrosylation, a reversible post-translational modification, has emerged as an important mechanism in cardiovascular biology, with critical relevance in the onset of proinflammatory agents-induced hyperpermeability (rewied by Dúran, IUBMB Life, v. 65, p. 819, 2013). This structural modification is based on the ability of nitric oxide (NO) to react with free sulfhydryl groups of cysteine residues (Lim, Chem. Res. Toxicol., v. 21, p. 2134, 2008). It has been demonstrated that the elevated production of NO during sepsis results in increased nitrosylation of the vascular tissue. Previous studies of our laboratory showed that protein S-nitrosylation is involved in the vascular dysfunction (cardiac failure, hypotension, hyporesponsiveness to vasoconstrictors and inadequate tissue perfusion, all linked to disease severity). The sulfhydryl-oxidizing agent 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), administered in rats submitted to cecal ligation and puncture-induced sepsis, after the condition was in full course, improved cardiovascular parameters (Benedet, BBA-Mol. Basis Dis., v. 1864, p. 307, 2018). However, since the impact of DTNB on the inflammatory component of sepsis was not assessed, this study aimed to investigate the effect of DTNB on inflammatory parameters in a pneumonia-induced sepsis model. **Methods:** Swiss female mice (35-40 g) were anesthetized by oxygen-isoflurane inhalation. An incision was made in the skin of the neck, the trachea was identified and 50 µL of bacterial suspension (Klebsiella pneumoniae, 1×10^8 CFU) was injected into the trachea with a sterile 30-gauge needle. Skin was sutured, warmed PBS was administered (30 mL/kg) and animals were left for recovery in a warm cage. Animals were treated with DTNB (12.5-50 mg/kg, s.c.) 12 h after infection. Animals were euthanized 24 h later. At 30 minutes before euthanizing, Evans blue dye (EBD), 40 mg/kg, was injected via gingival vein. Lungs and bronchoalveolar lavage fluid (BALF) were collected for pulmonary vascular permeability assay, leukocyte counts and NOx analysis. All results were expressed as mean ± S.E.M. and analyzed using oneway variance analysis followed by Bonferroni's post-hoc test. The difference between the means were significant when $p < 0.05$. All data were analyzed using GraphPad Prism® software version 5.01. **Results:** Sepsis increased vascular leakage in lung tissue (578.1 ± 57.2 µg EBD/g of wet tissue) when compared to naive group (79.1 ± 17.4 µg EBD/g of wet tissue). DTNB (25 mg/kg, s.c.) reduced vascular leakage (141.8 ± 31.2 µg EBD/g of wet tissue). BALF analysis showed that DTNB also reduced neutrophil migration in septic animals (2.02 ± 0.36 vs $0.69 \pm 0.19 \times 10^6$ cells/mL of BALF) and local NO production (6.4 ± 0.91 vs 3.4 ± 0.57 µM). **Conclusions:** Our results show that DTNB administration reduced inflammatory parameters associated with sepsis. These findings suggest that the DTNB beneficial effects in sepsis may be attributable, at least in part, to improvement in inflammation. **License number of ethics committee**: Research approval by Ethical Committee on Animal Use/UFSC: protocol 2627190617. **Financial support**: **Financial support**: CAPES and CNPq.

04.021 *In vitro* **oxidation of collagen promotes the formation of advanced oxidation protein products and the activation of human neutrophils**. Pereira GC[1], Bochi GV[1], Moresco RN[2] [1]UFSM – Fisiologia e Farmacologia, [2]UFSM – Ciências Farmacêuticas

Introduction: Protein oxidation is a process generally present during inflammation and the accumulation of advanced oxidation protein products (AOPP) has been related to the developed of several pathologies. Myeloperoxidase(MPO)-derived chlorinated oxidants produced by activated neutrophils contribute significantly to AOPP formation and human serum albumin (HSA) is considered the main protein responsible for the generation of AOPPs[1]. However, the molecular composition of AOPPs is unclear. Additional pathways and protein targets for AOPP formation are largely unknown. Thus, the aim of this study was investigate collagen as a potential source for AOPPs formation, and the effects of hypochlorous acid (HOCl)-treated collagen (collagen-AOPPs) on human neutrophils activity. It was also examined whether these effects can be counteracted by alpha-tocopherol. **Methods:** Under *in vitro* conditions, collagen was exposed to HOCl to produce collagen-AOPPs. The effect of collagen concentration on the level of collagen-AOPPs and the impact of alpha-tocopherol was evaluated by a concentration-effect curve. AOPP concentrations were measured by spectrophotometric assay[2]. The human neutrophils were isolated from the peripheral blood of healthy individuals after informed consent using a Ficcol-Histopaque density centrifugation method[3]. To examine the effect of collagen-AOPP on neutrophils, a suspension of neutrophils was incubated at 37ºC for 1 h with different concentrations of *in vitro*-prepared collagen-AOPP or unmodified collagen. Moreover, the effect of collagen-AOPP and alpha-tocopherol on apoptosis in neutrophils was measured by flow cytometry by the FITC Annexin V Apoptosis Detection Kit I. **Results:** Exposure of collagen to HOCl increased the levels of collagen-AOPPs. Collagen-AOPPs, when incubated with neutrophils, stimulated the production of AOPPs, nitric oxide (NO), superoxide radicals (O_2^-) and HOCl by these cells. Apoptotic events were also increased in neutrophils treated with collagen-AOPPs. Alpha-tocopherol prevented the collagen-AOPP formation and inhibited the collagen-AOPP-induced production of O_2^- and HOCl. In addition, the treatment of neutrophils with alpha-tocopherol reduced the number of apoptotic events when compared to cells exposed to collagen-AOPP only. **Conclusion:** These results suggest that collagen is an important protein that interacts with HOCl to form AOPPs and collagen-AOPP formation is linked to human neutrophil activation and cell death.

References: 1. CAPEILLÈRE-BLANDIN, C. BBA, v. 1689, p. 91, 2004. 2. WITKO-SARSAT, V. Kidney Int., v. 64, p. 82, 2003. 3. BOYUM, A. Tissue Antigens, v. 4, p. 269, 1974. **License number of ethics committee**: Comitê de Ética em Pesquisa com Seres Humanos, número 33649514.7.0000.5346. **Financial support**: Bolsas de estudo do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/Brasil) e da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/Brasil).

04.022 Ethanol consumption during adolescence increases hyperalgesia and impairs fever induced by lipopolysaccharide. Cruz JV, Maba IK, Correia D, Zampronio AR UFPR – Farmacologia

Introduction: Ethanol (EtOH) consumption, one of the main risks to the health worldwide, generally starts during adolescence in a binge (episodic consumption of high amounts) pattern. This is particularly important since in this critic period of life the central nervous system is going through changes to achieve maturation. There is evidence that binge EtOH consumption affects the innate and adaptive immune response including changes in the febrile response and hyperalgesia (Telles et al, Alcohol Clin Exp Res, 41: 507, 2017; de Oliveira et al., Pharmacol Biochem Behav 160: 63, 2017). The aim of this study was to evaluate simultaneously the febrile response and the hyperalgesia induced by lipopolysaccharide (LPS) in rats exposed to EtOH in a binge-like pattern during adolescence. **Methods:** Male Wistar rats were treated with an intraperitoneal (i.p) injection of EtOH (3g/kg, 25% w/v in saline) or saline on postnatal days (PND) 25, 26, 29, 30, 33, 34, 37, and 38. On PND 45, the animals received an intraperitoneal device to register body temperature. On PND 50, animals received the same dose of EtOH by oral route. Mechanical hyperalgesia assessed using an electronic analgesimeter, and body temperature were evaluated on PND 51, which is considered the early adulthood in the rat, at an ambient temperature of 28°C (thermoneutral zone). Additionally, to evaluate if cold-defense mechanisms were functional, the animals from both group saline and EtOH were submitted to a cold challenge, where the room temperature was kept at 15 ºC (CEUA/BIO-UFPR protocol #1120). **Results and Conclusion:** The administration of saline in both, EtOH- and saline-treated group did not induce significant changes in body temperature and mechanical threshold. LPS (50 µg/kg, i.p.) injection in saline-treated group induced a reduction on mechanical threshold from 46.5 ± 0.94 g to 37.4 ± 1.6 g and an increase in body temperature from 37.5 ± 0.15 °C to 38.4 ± 0.04 °C in the 4^{th} hour after injection. EtOH exposure during adolescence increased the LPS-induced hyperalgesia in 44% but abolished the febrile response. At an ambient temperature of 15 ºC, the animals exposed to EtOH did not show significant differences in the body temperature compared to saline-exposed animals. These results suggest that binge EtOH consumption during adolescence cause alterations in the febrile and nociceptive response to LPS, which persists till the early adulthood. These alterations affected fever and hyperalgesia in opposite directions as they increased the hyperalgesia while impaired the febrile response. The impairment in the febrile response does not result from a EtOH-induced deficiency in cold-defense mechanisms. **License number of ethics committee**: 1120 **Financial support**: CAPES e CNPq

04.023 Higher methylation observed in the second generation of intrauterine malnourished rats downregulate lung inflammatory response induced by LPS. Rodrigues LGA[1], Ramos APA[1], Balbino AM[1], Azevedo GA[1], Gil NL[1], Landgraf MA[1], Landgraf RG[1] - [1]Unifesp-Diadema – Ciências Farmacêuticas

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. We also investigated whether possible epigenetic changes could be involved in the inflammatory response observed in these animals. **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., *200□L). 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 DNA methylation, histone deacetylase (HDAC) and histone deacetylase 1 (HDAC1) by commercial kit. Western blot analysis was used to evaluate the protein expression of Cyclooxygenase 2 (COX-2) and 5-lipoxygenase (5-LO). **Results:** The malnourished group F2 (F2-UR) showed reduced total cell infiltration and neutrophils in the BALF after LPS instillation when compared to the offspring of nourished rats (NR). 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. Only the NR group showed increase in 5-LO expression. Basal methylation of the UR-F2 group was increased compared the control NR group, whereas only the UR-F2 group stimulated with LPS showed an increase in the DNA methylation when compared to its respective control. The activity of HDAC and HDAC1 was higher in the control NR group when compared to the control F2-UR group. There was no increase in HDAC activity after LPS stimulation in both groups. **Conclusion:** Our preliminary results indicate the participation of epigenetic mechanisms contributing to downregulate inflammatory response observed in the second generation of intrauterine malnourished rats. **License number of ethics committee**: Animal Research Ethical Committee: CEUA 1408220915. **Financial support**: FAPESP (2012/51104-8, 2017/02042-3) and CNPq.

04.024 Novel Histamine H_3/H_4 receptor antagonist LINS01005 and LINS01007 modulate lung inflammatory response in murine asthma model. Balbino AM[1], Lima LJS[1], Corrêa FM[1], Fernandes GAB[1], Landgraf MA[1,2], Fernades JPS[1], Landgraf RG[1]
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Introduction: Histamine is one of the most important chemical transmitters involved in several biological processes. The involvement of H_4R in experimental murine model has been demonstrated in several studies. 5-Substituted 1-[(2,3-dihydro-1-benzofuran-2-yl)methyl]piperazines (LINS01 series) molecules were synthesized. LINS01005 (*N*-phenyl-substituted compound) exhibitted low affinity for H_4R (pKi <5.0) while LINS01007 (5-chlorinated *N*-methyl compound) showed increased affinity for H_4R (pKi 6.06) and both showed antagonistic activity (*Corrêa MF, Front. Pharmacol. 8: 825, 2017*). In the present study we evaluated the effects of two novel histamine H_3R/H_4R antagonists in a murine asthma model. **Methods:** Histamine H_3R/H_4R antagonists LINS01005 and LINS01007 (3 mg/kg and 5 mg/kg) were given i.p. to ovalbumin sensitized mice 30 min before antigen challenge. After 24 h, bronchoalveolar lavage was performed for cell analysis and the lungs were removed for evaluation by western blot of cyclooxygenase-2 (COX-2),5-lipoxygenase (5-LO), factor nuclear kappa B (NF-kB) and signal transducer and activator of transcription 3 (STAT3) expression in C57Bl/6, 12 weeks old, male mice. **Results:** Treatment with LINS1005 caused a significant decrease in bronchoalveolar lavage cell numbers: total cells (107±16.5 to 20.05±6.5 $x10^4$/ml, p <0.05) and eosinophils (66.38± 20.7 to 18.25±5.2 $x10^4$/ml, p <0.05). Treatment with LINS01005 did not alter the cell infiltrate in bronchoalveolar lavage fluid at a dose of 3 mg/kg. Expression of COX-2, 5-LO, NF-κB and STAT3 in lung tissue was significantly reduced after treatment with LINS01007 at the dose of 3 mg/kg. Treatment with LINS01005 reduced the expression of these proteins only at the dose of 5 mg/kg. **Conclusion:** Our results showed a better anti-inflammatory activity of LINS01007 than compound LINS01005, suggesting the involvement of H_4R in this activity. Moreover, the compounds modulated the inflammatory cell infiltration and expression of COX-2 and 5-LO and activation of signaling pathways (NF -kB and STAT3) in murine asthma model. **License number of ethics committee**: Animal Research Ethical Committee: CEUA Nº 7601230317 **Financial support**: FAPESP (2012/51104-8, 2016/25028-3, 2016/23139-2, 2017/05441-6, 2017/02042-3) and CNPq (306480/2015-8; 455411/2014-0).

04.025 Evidence of anti-inflammatory activity of topically applied nanoencapsulated curcuminoids in comparison to curcuminoids *in natura*. Lima EP[1], Ames FQ[1], Sato F[2], Castro-Hoshino LV[2], Costa ACD[1], Sá IS[3], Gonçalves OH[3], Bersani-Amado CA[1] - [1]UEM – Farmacologia e Terapêutica, [2]UEM – Biofísica, [3]UTFPR – Medicamentos e Alimentos

Introduction: Numerous studies have shown that curcumin, extracted from curcuma longa, has antioxidant, antitumor, anti-inflammatory, anti-diabetic, antirheumatic, healing, antiviral and hepatoprotective activity. However, its use is limited due to its low solubility in water and low bioavailability when orally administered[1, 2, 3]. Recently, studies confirmed that curcumin encapsulation is a good alternative to improve its bioavailability and its efficacy. In these studies encapsulated curcumin was administered to the animals systemically. To our knowledge, to date, there are no reports demonstrating the anti-inflammatory effect of encapsulated curcumin (nanoparticles) when administered topically in experimental models of skin inflammation.

Aim: To evaluate: a) the anti-inflammatory activity of the curcuminoid nanoparticles (Nano-cur) compared to the curcuminoids in natura (Cur) when topically applied in an experimental model of cutaneous inflammation, and b) to determine its percutaneous penetration by Photoacoustic Spectroscopy (PAS). **Material and Methods:** Nanoparticles of curcuminoids conjugated to PVP and Cur were provided by the Food Technology Laboratory of the Federal Technological University of Paraná - Campo Mourão Campus. After applying croton oil (CO), groups of Swiss mice (n = 7) received a topical application of Nano-cur preparation or Cur (0.0625, 0.125, 0.25 e 0.5 mg/ear) on the left ear. The right ear received the vehicle that was used to dilute the croton oil (70% acetone). After 6 h, ear tissue was collected to determine the percent inhibition of edema (%), myeloperoxidase (MPO) activity and to perform PAS measurements. Data were analyzed using ANOVA - Tukey's test ($P < 0.05$). Experimental protocol was approved by Ethics Committee (ECAE/UEM 2624080318). **Results:** The treatment with Nano-cur preparation (0.125, 0.25 and 0.5 mg/ear) and Cur (0.25 and 0.5 mg/ear) reduced edema formation (59.36%, 89.30%, 76.29%, 66.13% and 63.10% respectively). The treatment with Nano-cur preparation (0.0625, 0.125, 0.25 and 0.5 mg/ear) and Cur (0.25 and 0.5 mg/ear) reduced myeloperoxidase activity (51.69%, 58.95%, 62.77 %, 53.74%, 55.60% and 54.88% respectively). The PAS analysis showed that topically curcuminoids in natura and curcuminoids nanoparticles penetrated into the tissue. **Conclusion:** The present results demonstrated the topical anti-inflammatory effect of the Nano-cur and Cur in a CO model of skin inflammation. However, the treatment with Nano-cur was more effective. PAS technique revealed the percutaneous penetration of the topically applied compounds. **References:** [1]ALMEIDA, M. et al. Food & function, v. 9(1), 440-449, 2018. [2]FACCHI S. P. et al. Int JBiolMacromol, v. 87, 237-245, 2016. [3]SUN, M. et al. J. Am. Chem. Soc., v. 135, 9099−9110, 2012. **License number of ethics committee**: ECAE/UEM 2624080318 **Financial support**: CNPq, CAPES e Fundação Araucária

04.026 Cecal ligation and puncture enhances fear generalization in rats surviving sepsis. Matias ME, Radulski DR, Silva TR, Stern CJ, Zampronio AR UFPR – Farmacologia

Introduction: Sepsis can be defined as a Systemic Immune Response Syndrome developed in response to an infection that reaches the circulatory system. This severe morbidity is associated with a high risk of mortality. Survivors constantly retain traumatic memories from the time spent at the Intensive Care Unit and are prone to develop Post-Traumatic Stress Disorder (PTSD). Consequently, individuals might present fear generalization, (i.e. the loss of memory specificity) therefore non-related events may elicit fear responses. Since the relation between sepsis and PTSD has not been explored, the objective of this study was to evaluate whether sepsis would induce PTSD-like outcomes. **Methods:** Wistar rats (180-250g) were submitted to the cecal ligation and puncture (CLP) procedure (1 and 3 punctures, groups CLP1 and CLP3, respectively) to induce sepsis or to sham surgery and received adequate post-surgery support. To make sure fear conditioning would not be affected by hyperalgesia promoted by systemic inflammation the paw withdrawal mechanical threshold of all groups was measured by electronic Von-Frey from day 1, up to day 8 after surgery. Ten days after surgery animals underwent a contextual fear conditioning protocol unable to induce fear generalization in the sham group. The protocol consisted of familiarization to Context A on day 10, fear conditioning on day 11 (Context A paired with three foot shocks of 0.6 mA/3 s), memory retrieval in Context A on day 12 and 19 (Test A1 and A2) and the test of memory specificity on day 13 and 20 when the animals were exposed to an unpaired Context B (Test B1 and B2). All sessions lasted 3 min. Freezing behavior was measured as an index of fear memory. One-way or two-way repeated measures ANOVA with either Bonferroni or Newman-Keuls post-hoc test was used to determine statistical significance between groups ($p < 0.05$). All experiments were approved by Animal Research Ethical Committee. **Results:** Two-way ANOVA showed that all groups had reduced mechanical withdrawal threshold one day following surgery. CLP1 had a lower mechanical threshold for up to 4 days and CLP3 for 6 days, after which mechanical threshold for all groups was not different from basal. All animals showed similar fear conditioning rates regardless of the surgery procedure. The one-way ANOVA showed that during Test A1 all groups presented similar freezing behavior (67.77 ± 3.69 %, 74.69 ± 4.35 % and 69.57 ± 3.44 % for sham, CLP1 and CLP3, respectively). However, during Test B1, freezing behavior was higher in both CLP1 (21.97.7 ± 3.43 %) and CLP3 (24.24 ± 3.80 %) compared to sham (8.18 ± 2.15 %). One week later only CLP3 showed an increased freezing behavior (Test B2: 30.19 ± 3.55 %) compared to the other groups (CLP1: 16.79 ± 4.62 % and sham: 10.40 ± 2.61 %). Importantly, there was no difference between sham and CLP1 in Test B2. **Conclusion:** CLP procedure causes hyperalgesia that persists for up to 6 days. Also, the CLP procedure increases the loss of memory specificity, an outcome associated with PTSD, which is dependent on the severity of the sepsis. **Financial support**: CAPES and Fundação Araucária. **License number of ethics committee**: 1019 **Financial support**: CAPES e Fundação Araucária

04.027 Evaluation of topical anti-inflammatory activity of oleic acid in skin inflammation models. Rocha FG, Ruziska RM, Brandenburg MM, Pawloski PL, Soley BS, Otuki MF, Cabrini DA UFPR – Farmacologia

Introduction: In a previous study, we verified that Moringa oleifera seed oil has anti-inflammatory activity for skin diseases when used topically. Popularly known as “moringa” or “acácia-branca” is a medicinal plant used worldwide for different diseases, including to alleviate inflammatory skin disorders. In the chemical composition of the seed oil the main compound present is the oleic acid (OA), which is an unsaturated long-chain fatty acid with demonstrated anti-inflammatory activity. However, there are no studies investigating the anti-inflammatory effect of OA. Also, being the major compound in the moringa oil, would it also be responsible for the topical anti-inflammatory effects of oil? **Methods:** Female Swiss mice were treated with Arachidonic Acid (AA, 2 mg/ear), phenol 10% or 12-O-Tetradecanoylphorbol-13-acetate (TPA, 2.5 µg/ear) to induce ear inflammation. Mice were divided into 5 groups: Naive, Control (AA, phenol or TPA), AO (20 µL/ear), Indomethacin (2 mg/ear) or dexamethasone (Dexa, 0.1 mg/ear). Ear thickness was measured using a digital micrometer as an index for edema formation. Animals from TPA experiment had biopsies of ear tissue collected for biochemical (enzymatic activity of myeloperoxidase -MPO) and histological analyses. The research was approved by Animal Research Ethical Committee. **Results:** CG-MS analysis showed a composition of 72.2% of AO in seed oil from *M. oleifera*. Topical application of AO reduced edema by 57.0 ± 0.2%, 59.2 ± 12.6%, 63.7 ± 1.1% in the AA, phenol and TPA models, respectively. Also, treatment with AO reduced MPO activity by 95.6 ± 0.3% and migratory inflammatory cell count by 67.2 ± 5.9%. Importantly, contralateral treatment with OA did not alter edema formation in the TPA model. **Conclusion:** Topical application of AO showed anti-inflammatory activity in acute models of skin disease, without evidence for systemic activity. These results suggest that OA is probably the main active compound with topical anti-inflammatory activity present in *M. oleifera* seed oil. Further studies are fundamental to confirm these effects of OA, including in chronic processes. Acknowledgement: CNPq, CAPES and INCT. **License number of ethics committee**: 1074 **Financial support**: CNPq, CAPES and INCT

04.028 *Moringa oleifera* **seed oil**: **Potential treatment for inflammatory skin diseases**. Brandenburg MM, Cretella ABM, Rocha FG, Pawloski PL, Soley BS, Cabrini DA, Otuki MF UFPR – Farmacologia

Introduction: Reports of the use of Moringa oleifera in traditional medicine point to its potential as resourceful pharmacological asset. Used as a nutritional component and in medicine, it has widespread use by the population of tropical countries. Mostly known for its effects on skin diseases, is commonly used via topical administration in different preparations. Therefore, the objective of this study is to evaluate the activity of the Moringa oleifera seed oil (OSMO) in skin inflammation models. **Methods:** Ear edema was induced with arachidonic acid (AA) 2 mg/ear, phenol 10% or TPA (2.5 µg/ear). Female Swiss mice were divided into groups: Naive, Control (AA, phenol or TPA), OSMO10 (1: 1 oil: acetone, 20 µL/ear), OSMO20 (20 µL/ear) and dexamethasone (0.1 mg/ear). The chronic inflammatory process was induced by multiple topical application of TPA (2.5 µg/ear) on alternate days for nine days. Treatment was applied topically twice a day from day 5 through 9. Ear thickness was evaluated with a digital micrometer. On the ninth day animals were euthanized and biopsies of ear tissue were collected and submitted to analyses (Myeloperoxidase (MPO), N-acetyl-β-D-glucosaminidase (NAG) and the histological analysis). Involvement of corticosteroid receptor on the anti-inflammatory activity of OSMO was evaluated by pre-treating animals with the antagonist mifepristone (50 mg/kg, s.c.) or vehicle, followed by topical TPA and treatment administration 30 min after and ear thickness measurements (6 h). The research was approved by Animal Research Ethical Committee. **Results:** Topical administration of OSMO was capable of reducing the edema formation on multiple models in different dosages. The OSMO20 showed a reduction of 34.5 ± 7.6%, 59.2 ± 12.6% and 69.2 ± 11.4% in ear thickens on the AA, phenol and TPA models, respectively. The topical treatment with different concentrations of OSMO also reduced the increase in MPO activity in 75.5% ± 0.4% for OSMO10 and in 95.6 ± 0.3% for OSMO20. In the histological analysis, OSMO was able to reduce the amount of migratory cells in a maximum of 67.60 ± 6.46 % for OSMO20. In the chronic inflammation model treatment with OSMO10 and OSMO20 reduced edema by 47.9 ± 9.1% and 47.1 ± 16.1%, respectively. The increased activity of both MPO and NAG were reverted by treatment with OSMO, with a maximum reduction of 89.8 ± 0.02% for MPO and 78.9 ± 0.02% for NAG for OSMO20. The migration of inflammatory cells and epidermis thickness were reduced by a maximum of 52.43 ± 2.02% and 17.53 ± 2.9% by OSMO20. In the evaluation of corticosteroid activity, topical treatment with OSMO20 reduced the edema formation by 61.93 ± 3.75% and pre-treatment with mifepristone reversed this effect. **Conclusion:** OSMO shows antiedematogenic activity when applied by topical administration in multiple models of skin inflammation. Its anti-inflammatory action seems to relay on a corticosteroid receptor pathway. Taken together, these results confirm its popular use and show that the OSMO has efficacy when used topically for the treatment of inflammatory skin diseases. Acknowledgement: CAPES, CNPq e INCT. **License number of ethics committee**: 1074 **Financial support**: CAPES, CNPq e INCT.

04.029 RANK/RANKL/OPG system in endotoxin-induced febrile response in female rats. Radulski DR, Matias ME, Zampronio AR UFPR – Farmacologia

Introduction: Changes in the signaling of the RANKL (Receptor Activator of Nuclear factor κB ligand), RANK (RANKL receptor) and OPG (osteoprotegerin, an endogenous blocker of RANKL) are involved in several diseases such as osteoporosis, cancer, rheumatoid arthritis and diabetes mellitus. Previous studies showed that this system is also involved in the febrile response induced by lipopolysaccharide (LPS) in male mice. The aim of this study was to evaluate if this RANK/RANKL signaling is involved in the febrile response induced by LPS in female rats and if it is affected by the female hormonal cycle. **Methods:** Female Wistar rats (180-220g) were submitted to ovariectomy or sham surgery. Male rats were also used as controls. Three weeks after surgery, animals received an intraperitoneal (i.p.) implant of a temperature recorder and when necessary an intracerebrovencular (i.c.v.) guide cannula. The experiments were conducted one week later and in sham-operated in Proestrus (PE, high estrogen levels), Diestrus (DE, low estrogen levels) or ovariectomized (OVX) female rats, due to the high and low estrogen blood levels, respectively. LPS (*E. coli*, 0111B4, 50 µg/kg, i.p.) was used as pyrogenic stimulus. When appropriate, animals were treated with OPG (2µg, i.c.v., 30 min before LPS) or with β-estradiol (E2, 10 µg/day, subcutaneously, for 5 days). After the experiments, the expression of RANK was evaluated in the hypothalamus by Western blot. All experiments were approved by the Institution's Animal Research Ethical Committee. **Results:** Sham-operated female rats in PE showed a lower core temperature during the light phase compared to males, DE, and OVX female rats. Intraperitoneal injection of LPS induced a pronounced febrile response in male and OVX female rats, and a significantly lower response in DE and PE female rats. Pre-treatment with the RANK antagonist, OPG, reduced the fever in males (1.54 ± 0.26°C vs. 0.63 ± 0.27°C), sham-operated females in DE (1.19 ± 0.27°C vs. 0.67 ± 0.26°C) and OVX female rats (1.84 ± 0.21°C vs. 1.09 ± 0.21°C) but not in sham-operated PE female rats. E2 replacement in OVX animals reduced LPS-induced fever similarly to OPG treatment (1.96 ± 0.21°C vs. 1.07 ± 0.21°C). However, the combination of both, E2 and OPG treatment did not further modify the effect observed with any individual treatments. Western blotting analysis demonstrated that PE females exhibit an increased RANK expression (55.7%) after LPS injection while the other groups did not show a significant increase in the expression of this receptor in the hypothalamus. Moreover, E2 replacement in OVX rats was also capable of increasing the expression of RANK (42.9%). **Conclusion:** These results suggest the RANK/RANKL/OPG system participates in LPS-induced fever in DE and OVX female rats but not when the fever is induced during PE. The lack of involvement of this system in PE is not related to a reduced expression of RANK in the hypothalamus. On the contrary, the estrogen, which is released during this phase, seems to increase the expression of RANK in the hypothalamus. **License number of ethics committee**: 1021 **Financial support**: CAPES and CNPQ

04.030 Periodontal status and metabolic changes in rats submitted simultaneously to chronic administration of atypical antipsychotics and experimental periodontitis. Soares MA[1], Martins AF[1], Silva NLC[2], Matias DO[1], Miranda ALP[2], Tributino JLM[3], Alves LM[3] - [1]UFRJ, [2]UFRJ – Ciências Farmacêuticas, [3]UFRJ – Ciências Biomédicas

Introduction: Periodontal disease (PD) represents a group of inflammatory diseases that affect the tissues of support and fixation of tooth. Epidemiological studies have demonstrated higher incidence and severity of PD in patients with metabolic alterations as well as delimiting these alterations as a risk factor for PD development in several populations (Kaye, E. K., J. Dent. Res., v. 95, p. 822, 2016). One of the major triggers of metabolic changes is the use of medications, including the antipsychotics olanzapine (OLA) and clozapine (CLO), multi-target drugs associated with occurrence of metabolic syndrome in 50% of users (Baptista, T. Metab. Syndr. Relat. Disord., v.2, p. 290, 2004). Considering the importance of evaluating interactions between metabolic changes induced by atypical antipsychotics and the inflammatory process caused by periodontal disease, this study **Aims:** to evaluate whether OLA- and CLO-induced metabolic alterations exacerbate the experimental periodontitis in rats. **Methods:** Female Wistar rats were divided into the following groups: VEH (0.9% NaCl, 0.1 mL/20 g p.o., once a day), OLA (olanzapine 1.5 mg/kg p.o., twice a day), CLO (clozapine 21 mg/kg p.o., once a day), VEH + PD (NaCl 0.9% + PD induced on day 30) and OLA + PD (olanzapine 1.5 mg/kg p.o., twice a day + induced PD on the 30th day). The animals had their food intake and body weight monitored every 3 days and received daily NaCl, OLA or CLO for 45 days, and in the VEI + DP and OLA + DP groups, PD was induced by bilateral ligature of first mandibular molars on the 30th day. The glucose tolerance (GTT) and insulin resistance tests were performed on 33rd and 42nd days, respectively. At the end of experimental period, animals were euthanized and had their jaws, gingiva, blood or plasma and adipose tissue collected for evaluation of metabolic and inflammatory parameters. **Results:** animals from the OLA group showed a significant weight gain in relation to the VEH control starting from the 21st day, with an increase of 35.18% of the total body mass evaluated by representative area under curve of the 45 days of intervention, without changes in food intake, while animals in CLO group had a reduction of 30.67% in adipose tissue in relation to VEH group. The administration of OLA and the establishment of PD increased the glycemia of the animals immediately after the glycemic challenge in GTT, and animals in the OLA group had a blood glucose increase of 42.29% versus 23.28% of VEH animals relative to day 0. The groups OLA + DP and CLO showed an increase in gamma-glutamyl transferase. The antipsychotics were not able to increase mandibular alveolar bone loss of these animals; however, an 88% reduction of MPO in the gingival tissue was observed in CLO group, with no alterations in the neutrophil count in blood, suggesting alterations in the activity of these cells in the periodontal tissue. The same group showed an increase of 118.40% in gingival TNF concentrations. **Conclusion:** this work corroborates data from literature related to the occurrence of metabolic alterations in animals caused by the chronic administration of antipsychotics and shows for the first-time alterations in inflammatory markers in periodontum induced by chronic administration of clozapine. **License number of ethics committee**: CEUA/UFRJ 057/2018 **Financial support**: CAPES/FAPERJ

04.031 Fourth-generation immucillin as a new strategy for multiple sclerosis: **pre-clinical evidence**. Silva RBM[1,2], Bergo PHF[3], Machado P[2], Rodrigues-Junior VS[2,1], Campos MM[1,2,4] [1]PUCRS – Toxicologia e Farmacologia, [2]PUCRS –Tuberculose, [3]PUCRS –Medicina, [4]PUCRS – Ciências da Saúde

Introduction: Multiple sclerosis (MS) is the most prevalent chronic inflammatory disease of the central nervous system, affecting 2.3-million people worldwide. Tissue damage in MS results from a complex interplay among the immune system, glia and neurons (Reich, N Engl J Med, 378(2): 169, 2018). The fourth-generation immucillin (DI4G) selectively inhibits the purine nucleoside phosphorylase enzyme (PNP), which in turn, is expressed by immune cells (Ho, Proc Natl Acad Sci, 107(11): 4805, 2018). This study investigated the effects of DI4G in a mouse model of multiple sclerosis, in comparison with fingolimod - a drug clinically used for MS management. **Methods:** Experimental autoimmune encephalomyelitis (EAE), a classical MS model, was induced in female C57BL/6 mice (18-22 g, 6-8 weeks old, N=10/group), by a subcutaneous 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 (intraperitoneal) on days 0 and 2 post-immunization. Clinical signs were measured 7 days post-immunization, every 2 days, over a total period of 25 days. Tactile and thermal hypersensitivity were evaluated using von Frey filaments and the hot-plate test, respectively. The rotarod test was used to analyze the motor coordination, whilst the spatial memory was assessed using the object location task. An automatic open-field system was used to evaluate the spontaneous locomotor activity. Mice were monitored daily and were weighted every 5 days (as parameters of health), for up to 25 days. The spleens were weighted as indicative of immune cell production. Animals were treated with DI4G (2.5 mg/kg, intraperitoneal) or fingolimod (0.3 mg/kg, oral route), from seven to 25 days after the onset of EAE induction. **Results:** The mechanical and thermal hypersensitivity elicited by EAE induction was reversed by DI4G and fingolimod (32 ± 8% and 38 ± 10%; 23 ± 6 and 22 ± 3, respectively). The administration of DI4G or fingolimod produced a marked decrease of clinical scores (50 ± 4% and 45 ± 4%, respectively), allied to an improvement of mouse motor coordination (30 ± 8%), spatial memory test (34 ± 7%), ambulatory movement (60 ± 9%) and travelled distance (71 ± 13%) - exclusively for DI4G treatment, when compared to the vehicle group. The treatment with DI4G and fingolimod also prevented the body weight loss, at the 10^{th} and 15^{th} day. DI4G significantly reduced the EAE-elicited splenomegaly (26 ± 4%), whereas fingolimod failed to display any improvement of this parameter. **Conclusion:** This data brings novel evidence indicating that pharmacological modulation of PNP by DI4G greatly improves the symptoms and signs in a mouse model of MS. This might represent a promising strategy for managing MS in a near future. Additional studies are currently in development to further evaluating relevance of PNP in the progress of MS. **License number of ethics committee**: CEUA-PUCRS: 14/00424 **Financial support**: CAPES, INCT-TB, CNPq, PUCRS

04.032 Role of calcineurin phosphatase in the sepsis pathophysiological events

Borges VF[1], Castanheira FVS[1], Kanashiro A[1], Silva CMS[2], Wanderley CWS[2], Hiroki C[3], Lima MHF[3], Schneider AH[1], Cunha FQ[1] [1]FMRP-USP – Farmacologia, [2]UFC – Farmacologia, [3]FMRP-USP – Bioquímica e Imunologia

Introduction: calcineurin is a calcium/calmodulin-activated serine-threonine phosthase that promotes T cell activation and proliferation through the IL-2 production. The literature has few papers that look for the role of calcineurin and related pathways in the innate immunity. However, the calcineurin inhibitors treatment, very common as immunosuppressants for prevention of transplants rejection, have been reported to increase the patient susceptibility to infections, mainly that caused by fungi. The aim of this study was to investigate the calcineurin role in the sepsis associated pathophysiological events. **Methods:** wild type C57/BL6mice were treated with the calcineurin inhibitor FK-506 and submited to cecal ligation and puncture (CLP) sepsis model. Besides the survival curve, other sepsis parameters were obtained, such as cell migration, bacteremy and levels of citokines and injury biochemical markers, as well mean arterial pressure and heart rate. **Results:** mice treated with FK-506 presented lower survival to CLP. The FK-506 related susceptibility is associated with: reduced neutrophils migration to the peritoneal cavity; increased number of bacteria in the blood; more pronounced reduction of CXCR2 chemotactic receptor expression in circulating neutrophils; elevated inflammatory response; higher injury markers and bradycardia. **Conclusion:** the calcineurin activity is important to neutrophil migration and the infection control in polymicrobial sepsis. Our work confirms the calcineurin importance in the innate immunity. **License number of ethics committee**: 002/2013-1 **Financial support**: FAPESP, CAPES, CNPq, CRID.

04.033 Strontium ranelate inhibits osteoclastogenesis and osteoclast activity. Lima V[1,2], Taira TM[3], Fukada SY[4], Cunha FQ[2] [1]UFC – Physiology and Pharmacology, [2]FMRP-USP, [3]FMRP-USP – Pediatric Dentistry, [4]FCFRP-USP – Physics and Chemistry

Introduction: Strontium ranelate (SrR) is an anti-osteoporotic drug that acts by a dual mechanism of action, by inducing the bone formation and inhibiting the bone resorption. The SrR have been reported to increase osteogenic functioning in mesenchymal stem cells. However, some aspects of their effects on osteoclastogenesis remain unclear. We evaluated the SrR effects on the NFATc1 and DC-STAMP signaling axis in the osteoclast multinucleation process. **Methods:** Bone marrow-derived macrophages from a C57BL/6 mouse were cultured with M-CSF, RANKL and SrR (0.1 mM, 0.3 mM, and 1 mM) for multinuclear tartrate-resistant acid phosphatase (TRAP)-stained cells, the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-based cytotoxicity assay, qPCR for bone markers, and pit osteoassay. The Committee on Ethics in Animal Experimentation in the University of Sao Paulo (Ribeirao Preto, Brazil) approved the protocols (215/2017). **Results:** The *in vitro* incubation of primary osteoclast cultures with SrR decreased RANKL-induced osteoclast differentiation without affecting cell viability. SrR inhibits osteoclast formation through the downregulated the mRNA expression of the nuclear factor activated T-cells, cytoplasmic 1(Nfatc1), a master regulator of osteoclast formation, and by the downregulation of cathepsin K (CtsK) from 48h of culture, which is important to the osteoclast. Moreover, SrR downregulated the dendritic cell-specific transmembrane protein (DC-STAMP), TRAP, and matrix metalloproteinase (MMP)-9 for 24-72h. These effects were supported by inhibition of pits formation activities. **Conclusion:** Our data show that SrR presented antiresorptive action by inhibition of the pre-osteoclast fusion and consequent the activation of osteoclasts. **License number of ethics committee**: 215/2017 **Financial support**: National Council for Scientific and Technological Devellopment (CNPq: 110935/2016-0), Center for Research in Inflammatory Diseases (CRID/São Paulo Research Foundation (FAPESP: 2013/08216-2) and Regular Project (FAPESP: 215/09034-0)

04.034 AT1 receptor antagonist modulates gene expression of Renin-Angiotensin System components and bone metabolism markers and attenuates bone loss in rats with experimentally-induced periodontal disease. Silva GP, Dionisio TJ, Parisi VA, Garbieri TF, Colombini-Ishikiriama BL, Santos CF FOB – Ciências Biológicas

Introduction: The renin-angiotensin system (RAS) is known for its role in cardiovascular regulation, but it has been implicated as an important factor in inflammatory processes. The aim of this study was to evaluate the effect of AT1 receptor blockade on the progression of experimentally-induced periodontal disease (PD) in rats, by evaluating gene expression (q-PCR) and bone loss analysis. In addition to the RAS-regulating genes, gene expression of bone markers metabolism in the mandible around the first lower molar affected by PD was evaluated. **Methods:** After anesthesia, silk suture thread (4.0) was placed around the lower right first molar. This method is well established and is capable of causing alveolar bone loss in addition to compromising adjacent supporting tissues. Animals remained with the silk suture wire around the tooth for 1, 3, 7 and 14 days. For bone loss analysis, the rats' jaws were stained with 1% methylene blue, photographed and the area of bone loss around the lower first molar was evaluated with ImageJ software (version 1.48, USA). Besides treatment with water and losartan (30 mg/kg/day) on the same day of PD induction, groups of animals were previously treated with the same drug and at the same dosage for 30 days. Therefore, the present study contained 4 groups with 5 animals in each group: G1 - control without PD; G2 - animals with PD and treated with water; G3 – losartan-treated animals (treatment started in the same day of PD induction) and G4 - animals previously treated with losartan for 30 days followed by induction of PD and continuity of treatment. **Results:** The results revealed that water-treated animals (G2) had greater bone loss after 14 days of PD compared with control group (G1) and with losartan-treated rats (G3 and G4). Moreover, PD promoted AT1a receptor increased expression, but losartan treatment did not modulate this response. On the other hand, PD alone did not influence the expression of ECA-2 enzyme, however losartan treatment promoted an increase in MAS and AT2 receptors expression, which may explain the decrease of bone loss observed in the animals treated with this AT1 receptor antagonist, since ECA-2 is capable of cleaving Ang II into Ang 1-7, which in turn promotes anti-inflammatory actions when bound to MAS receptors. The same anti-inflammatory mechanism occurs when Ang II binds to AT2 receptor. The results of bone formation/absorption markers also help to explain the decrease of bone loss in losartan-treated rats since it was possible to observe that RANKL levels increased with PD, however losartan treatment prevented this increase. In addition, Phex expression was higher in losartan-treated animals after 7 days of PD compared with water treated animals. **Conclusion:** The present results support the conclusion that AT1 receptor modulates PD progression. **License number of ethics committee**: 020/2016
Financial support: FAPESP 2015/03965-2

04.036 Effects of Telmisartan and Losartan on DNA fragmentation and oxidative stress of germ cells in irradiated testes. Spadella MA[1], Mansano NS[2], Santos CR[1], Chies AB[3] - [1]FAMEMA – Morfologia, [2]USP – Ciências Biomédicas, [3]FAMEMA – Farmacologia e Terapêutica

Introduction: The ionizing radiation-induced oxidative stress may injury tumoral and healthy tissues in radiotherapeutic procedures. Evidences suggest the involvement of the Renin-Angiotensin System in this redox imbalance. Testes are particularly radiosensitive, thus, the spermatogenesis can be significantly affected by radiation. **Objectives:** This study investigated whether AT_1 receptor antagonists minimize radioinduced damage on male reproductive tissue. **Methods:** Male *Wistar* rats distributed in six groups: 0 Gray (Gy) (control), 5Gy (single dose in the scrotum), Telmisartan (12mg/kg-1x/day), 5Gy+Telmisartan, Losartan (34mg/kg-2x/day) and 5Gy+Losartan. The treatment for 60 days started on the day after irradiation. Testes were processed for histopathological analysis. The DNA fragmentation was verified by the TUNEL method. Imunolocalization for NOX-5 protein was also performed. **Results:** Seminiferous tubules were disorganized, with significant structural impairment in both untreated and treated irradiated testes. The seminiferous epithelium was depleted, with severe lack of germ cells and presence of vacuoles. Telmisartan and losartan did not significantly attenuate these histopathological damages. However, several seminiferous tubules with signs of regeneration were founded in treated animals. TUNEL-positive nucleus were detected in surviving Sertoli cells and spermatogonia from irradiated rats. In irradiated groups, that were also treated, TUNEL labelling was still evident in surviving cells. Furthermore, lesser quantity of germ cells with DNA fragmentation was observed in regenerate tubules. NOX-5-positive cells were detected mainly in spermatocytes and spermatids from seminiferous tubules in control groups. In irradiated rats, NOX-5 was detected in surviving spermatogonia and Sertoli cells. In irradiated animals that received telmisartan and losartan, the labelling was also observed in spermatocytes and spermatids from regenerated seminiferous tubules. **Conclusions:** The results demonstrated a beginning of reducing in the severity of the histopathological damages, as well as, an apparent reduction in the DNA fragmentation of male germ cells in restored tubules by AT1 receptor antagonists treatment. It was expected an increase of NOX-5 expression in the irradiated testes, that could be related to the radiation-induced oxidative stress. However, in consequence of the intense cell loss in the seminiferous tubules in irradiated groups, this increment could not be observed. On the other hand, the expression of NOX-5 in testes was also evidenced in control groups, since under normal physiological conditions, it is suggested that it acts in stages of spermatogenesis.
License number of ethics committee: This study was approved by the institution?s Animal Experimentation Ethics Committee (CEUA/Famema, protocol number 552/14).
Financial support: This work was supported by Brazilian agency FAPESP - Fundação de Amparo à Pesquisa do Estado de São Paulo [grant number 2015/20740-4].

04.037 Regulatory role of cytochrome P450 pathway in skeletal muscle degeneration and regeneration phases induced by phospholipase A_2 isolated from a snake venom. Damico MV[1], Zuntini ACS[1], Fortes-Dias CL[2], Moreira V[1] [1]Unifesp – Farmacologia, [2]FUNED – Pesquisa e Desenvolvimento

Introduction: Eicosanoids are bioactive lipid mediators, which promote a variety of regulatory mechanisms under physiological and pathophysiological conditions. In this context, the functions and presence of these inflammatory lipid mediators derived from the metabolism of arachidonic acid (AA) mainly prostaglandins and some leukotrienes have been described to be involved mainly in the proliferation of muscle cells and regulation of inflammatory response present during skeletal muscle regeneration after injury. However, the influence of another group of lipid mediators, such as hydroxy-eicosatetraenoic acid (HETE) and epoxides (EET) derived from the cytochrome P450 (CYP450) pathway have never been investigated on the degeneration and muscle regeneration, in vivo experimental models. The aim of this study was to analyze the regulatory effects of eicosanoids produced by CYP450 pathway on histological aspects of skeletal muscle degeneration and regeneration induced by a phospholipase miotoxin from viperidae snake venom. **Methods:** Male Swiss mice (20g) were injected in gastrocnemius muscle (i.m) with crotoxin basic (CB) isolated from Crotalus durissus terrificus snake venom (37,5µg/kg/50µL) or saline solution (SS). After 30 min and 48 h of injection of CB or saline SS, distinct groups of mice received oral administration (p.o.) of SKF-525A (SKF), a CYP450 non-specific inhibitor compound (25 mg/kg/100µL)1 or vehicle (CMC 1%). After 6 and 24h or 3, 7, 14 and 21d from i.m. injection, mice were sacrificed by cervical displacement and gastrocnemius muscles were dissected and fixed in 4% paraformaldehyde. The tissue was dehydrated, embedded in paraffin and sections (4 µm) were stained with hematoxylin/eosin and analyzed by microscope (Nikon Eclipse E800). Percentage of injured fibers (%IF), basophilic fibers (%BF) and central nuclei fibers (%CN), were calculated from total muscle fibers. The counting of cell influx (CI) and diameter of central nuclei fibers (DCN) into local of injury and regeneration phases were also analyzed histological tissue. **Results:** In degenerative phase (6h), mice treated with CB/SKF showed significant increase ($p<0,05$) of %FL (42,8±2,0%) when compared to group CB/CMC (25,4±3,4%). After 3d, muscles of animal treated with CB/SKF presented significantly increase ($p<0,05$) of %BF (64,7±2,2%) when compared to CB/CMC-treated mice (54,7±0,9%). In regenerative phase (21d), mice treated with CB/SKF showed significant increase ($p<0,05$) of DCN (1.131±26µm^2) when compared to mice treated with CB/CMC (853±70µm^2). **Conclusion:** For the first time, the obtained data suggest that CYP450-derived mediators are produced in local after muscular tissue injury and that down-regulate elements involved in the phase of muscular degeneration, such as necrosis. Likewise, during the regeneration process these groups of lipid mediators down-regulate activated molecular components involved in the regeneration phase, characterized by fusion and growing of myofibers processes. 1 ZABRODSKII, P. F. et al. Bull Exp Biol Med, v. 142, p. 324, 2006. **License number of ethics committee**: Ethics Committee in Animal Experimentation: 4892220616 **Financial support**: FAPESP; CNPq

04.038 TRPV1 contributes to the development of cerebral malaria by modulating the integrity of the blood-brain barrier and oxidative stress in mice. Pereira DMS[1], Teixeira SA[2], Murillo O[3], Peixoto EPM[3], Araújo MC[1], Sousa NCF[1], Monteiro-Neto V[4], Calixto JB[5], Cunha TM[6], Marinho CRF[3], Muscará MN[2], Fernandes ES[1] [1]Ceuma – Imunofarmacologia, [2]USP – Farmacologia, [3]USP – Parasitologia, [4]Ceuma – Microbiologia, [5]CIEnP – Farmacologia, [6]FMRP-USP – Bioquímica e Imunologia

Malaria is an infectious, parasitic, systemic and non-contagious disease with great morbidity and mortality; affects 216 million individuals around the world, reaching approximately 429,000 deaths/year (WHO, 2016). Cerebral malaria (CM) is an important lethal encephalopathy associated with brain inflammation and related to the development of irreversible neuronal sequelae. The mechanisms of the host´s immune response to the *Plasmodium* play a decisive role in the clinical progression of CM and, therefore, influences the outcome of the disease. We recently shown that lack of transient receptor potential vanilloid 1 (TRPV1), a Ca^{+2} permeable ion channel expressed on neuronal/non-neuronal cells, protects against cerebral syndrome induced by *P. berghei* ANKA in C57BL/6 mice (Pereira et al., 2017). However, the mechanisms involved in this response are unclear. Thus, we investigated whether TRPV1 ablation influences blood-brain barrier (BBB) permeability and the plasma levels of oxidative stress markers in murine CM. For this, TRPV1 wild type (WT) and knockout (KO) mice received an intraperitoneal (i.p.) injection containing *P. berghei* ANKA ($1x10^6$ infected red blood cells). Parasitaemia, body weight, signals, symptoms and mortality were evaluated daily/14 days post-infection. In another set of experiments, animals were culled as soon as the disease progressed to the stages III/IV of disease and their brain and plasma were collected for analysis of plasma extravazation, expression of the endothelial tight junction biomarkers JAM-A and claudin 5, plasma H_2O_2, nitrotyrosine and carbonyl protein residues. Parasitaemia was similar irrespective of genotype; however, TRPV1KO exhibited higher survival rates and less signals and symptoms of disease than WT mice. Infected WT mice presented enhanced plasma extravasation and mRNA expression of JAM-A and claudin-5 in comparison to KO mice. Alternatively, KO animals presented higher concentrations of H_2O_2 and increased nitrotyrosine and carbonyl protein residues in their plasma than infected WT. Overall, these data indicate that TRPV1 contributes to the development and outcome of CM by regulating the integrity of the BBB and the systemic oxidative stress products. We suggest that TRPV1 may be an important target to treat neurological disorders in which oxidative stress and disruption of the BBB play a role. **Keywords:** Cerebral Malaria. Oxidative stress. Blood Brain Barrier. TRPV1 **Financial support**: CAPES, CNPq, FAPEMA, FAPESP. Ethics Committee approval 58/12. **License number of ethics committee**: 58/2012 **Financial support**: CAPES, CNPq, FAPEMA, FAPESP

04.040 Gestational malnutrition induced lower expression of TLR4 and ObR in mice offspring. Balbino AM[1], Ramos APA[2], Gil NL[2], Silva MM[2], Azevedo GA[2], Landgraf MA[3], Landgraf RG[4] [1]Unifesp-Diadema – Ciências Farmacêuticas, [2]Unifesp-Diadema – Ciências Farmacêuticas, [3]Uninove – Ciências Farmacêuticas, [4]Unifesp – Ciências Farmacêuticas

Inadequate nutrition in pregnancy results in consequences for fetus. This condition is associated more susceptibility to infectious diseases in offspring life as a result of alterations in the immune response. Respiratory diseases are common by world, but lung has mechanisms to fight immunogen such as ciliated epithelium, production of mucus, resident macrophages in airways, pattern recognition receptors present in cells surface trigger innate immune response. However, fetus immune system response caused by maternal protein calorie restriction during pregnancy is a few known. This work we examined the acute lung inflammation of C57Bl6 mice borned in protein calorie restriction environment during pregnancy. In this study pregnants C57Bl6 females were separated into two groups: Control Group (CG) and Restrict Group (RG). The CG female were fed ad libitum chow and RG females RG were fed same chow at 30% of CG female intake. After parturition ever, dams received ad libitum chow. The male offspring mice with 100 days of age received lipopolysaccharide LPS (45ug) or saline (intranasal). After 6 hours the animals were euthanized and collected lavage bronchoalveolar (for total cell count and neutrophils) and pulmonary tissues to analyses the expression of the TLR4 and production proinflammatory cytokines. In RG mice when stimulated with LPS has been observed a reduction in inflow of inflammatory cells, diminished TLR4 and ObR expression and increased production proinflammatory cytokines compared to the CG mice in same conditions. In this study we observed that LPS failed in activate TLR 4 pathway in RG mice causing a reduced phagocytes migration to inflammatory site. This way immunogen has not been fought with same efficiency of CG mice and consequently its presence maintains the production proinflammatory cytokines like IL 1b and TNF a. Our results indicate intrauterine protein-calorie restriction decreased the immune response in these animals. **License number of ethics committee**: 5896160117 **Financial support**: FAPESP

04.041 TNF-α reduces ADP- and thrombin-induced platelet aggregation via PKCε, AP-1 and IKK activation. Bonfitto PHL, Marcondes S FCM-Unicamp – Farmacologia

Introduction: Platelets have been described as important cells in inflammation, however, the effects of cytokines on platelet reactivity are rarely studied. Our group observed that TNF-α 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. The objective of the present work is to determine the signaling pathways 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 (1-3000pg/ml) for 15 or 30min before ADP (5μM) or thrombin (200mU) addition. In some experiments the platelets were incubated with the inhibitors of PKCδ (rottlerin, 5μM), PKCε (SC3095, 1μM) or the unspecific PKC inhibitor (GF109203X, 10μM), PI3K (LY294002, 25μM), Akt (API-1, 20μM), c-Src (PP2, 10μM), ERK (FR180204, 30μM), p38MAPK (SB203580, 1μM), JNK (SP600125, 10nM), AP-1 (T5224, 20μM) or IKK (IKK16, 0,1μM) for 5min before TNF-α addition. Akt Ser473 phosphorylation on TNF-α-treated platelets was measured through western blotting. The effect of IKK16 on TNF-α-incubated platelets on calcium mobilization was investigated through fluorescence assays using fluo-3AM. **Results:** TNF-α (15min) reduced both thrombin and ADP-induced platelet aggregation *in vitro*; it was also observed that the concentration of 100pg/ml led to the major inhibition of aggregation (36,63% ± 7,7 of inhibition). The general PKC inhibitor (GF109203X) or of PKCδ (rottlerin) did not affect the inhibition of platelet aggregation induced by TNF-α. However, inhibition of PKCε (SC3095) partially diminished the effect of the cytokine (32,26% of reduction). Inhibition of PI3K (LY294002) did not modify the inhibition of platelet aggregation induced by TNF-α. The cytokine increased ADP-induced Akt Ser473 phosphorylation; however Akt inhibition (API-1) did not prevent the inhibitory effect of TNF-α. The inhibition of c-Src (PP2), ERK (FR180204), p38MAPK (SB203580) and JNK (SP600125) also did not diminish the inhibitory effect induced by the cytokine. However, inhibition of AP-1 (T5224) and IKK (IKK16) significantly reduced the TNF-α-induced inhibition of platelet aggregation (reduction of 43% and 40,35% respectively). TNF-α did not affect platelet calcium influx, however it reduced the internal calcium mobilization, which was abrogated by the IKK inhibitor (IKK16). **Conclusion:** TNF-α dose-dependently inhibits both thrombin and ADP-induced platelet aggregation which is accompanied by reduction of internal calcium mobilization. This effect is independent of Akt phosphorylation and PI3K, c-Src, ERK, p38MAPK, JNK and all PKC isoforms, except PKCε, activation. However, PKCε activation as well as IKK and AP-1 activities are involved with the reduction of platelet aggregation induced by TNF-α. **License number of ethics committee**: 3709-1
Financial support: CNPQ

04.043 Protease Activated Receptor (PAR) 2 contributes to inflammatory response mediated by the activation of Toll Like Receptor (TLR)4 in murine peritoneal macrophages. Barra A, Navia-Pelaez JM, Capettini LSA, Klein A UFMG – Farmacologia

Introduction: PAR2 is a G protein-coupled receptor activated by the proteolytic cleavage of its extracellular N terminus by trypsin-like enzymes and expressed on the surface of immune cells, such as macrophages. Previous studies described synergistic PAR2 and TLR4 mediated response inflammatory. However the effect of the interaction between these receptors in macrophage-mediated phagocytosis and macrophage activity are still unclear. **Aim:** We investigate the interaction between PAR2 and TLR4 *in vitro* phagocytosis of zymosan and on inflammatory mediators production in the peritoneal macrophages. **Methods:** Macrophages obtained from thioglycolate-injected wild or TLR4 (-/-) C57BL/6 mice were preincubated with lipopolysaccharide (LPS,10ng/mL) 45 minutes prior to PAR2 agonist SLIGRL-NH_2 (SLI, 30µM) or tryptase (TRYP, 10^{-8}M). The incubation occured in the presence or not with their antagonist ENMD-1068 (ENMD, 0.1µM), followed by incubation with zymosan (Zy, 10 µg/ml, 1h). Phagocytosis was assessed as percentage of phagocytic cells (PP), mean number of particles per cell (MNP) and as phagocytic index (PI) determined as PI = PP x MNP. The nitric oxide level (NO) was measured by Griess method and Reative Oxygen Species (ROS) by chemiluminescence after 24 hours of stimulation with LPS and/or SLI (30µM) or both. Measurement level of calcium reticular was detected using flow cytometry. Statistical analyses were performed using one-way ANOVA followed by Tukey post-test. **Results:** SLI incubation increases PI in macrophage from wild mice (DMEN, 0.4500* ± 0.04041; LPS, 1.380 ± 0.03055; LPS+SLI, 2.660** ± 0.2718; LPS + TRYP, 2.050* ± 0.1504), and pre-incubation with ENMD reduced PI in LPS-stimulated macrophage (DMEM, 1.407* ± 0.1345; LPS, 1.940 ± 0.1015; LPS+ENMD, 1.200** ± 0.1845; LPS + TRYP, 2.050* ± 0.1504), ***p*<0.001 and **p*<0.05 when compared to LPS. SLI increases nitrite production in LPS-stimulated macrophage (µM) at 24h of the culture in macrophage from wild mice (DMEM, 1.78 ± 0.067; SLI, 1.98 ± 0.13; LPS, 6.47 ± 0.28; LPS + SLI, 9.05* ± 0.65), *p*<0.001 when compared to LPS only. However not increases ROS (RLU units) at 24 h (DMEM, 11888 ± 182.4; ZYM, 55249 ± 1777; LPS, 15975 ± 710.6; SLI,11938 ± 225.8; LPS + SLI, 16910 ± 405.8). SLI increases the release of reticular calcium (DMEM, 1.357 ± 0.008; SLI, 1.125 ± 0.009; LPS, 1.035 ± 0.014.; LPS + SLI, 1.018* ± 0.008), *p*<0.001 when compared to SLI only. **Discussion:** *In vitro* PAR2 activation was able to increase phagocytosis in LPS-stimulated peritoneal macrophages, in addition, was able to increases the NO production and reticular calcium release, but not ROS. **Conclusion:** Taken together our results suggest a role for PAR2 and TLR4 modulating importants mechanisms of macrophage repertory**.** **Financial support:** CNPq, Fapemig. Experimental procedures were approved by the local animal ethics committee (certificate number 150/2017). **License number of ethics committee**: 150/2017 **Financial support**: Fapemig

04.044 Staphylococcal enterotoxins Type A (SEA) and B (SEB) induces neutrophil dysfunction by a mechanisms dependent on MHC-Class II activation Ferreira-Duarte AP[1], Santos-Ramos V[1], Pinheiro-Torres AS[1], Antunes E[2], DeSouza IA[1] - [1]Medicina de Jundiaí - FMJ - Biologia e Fisiologia, [2]Unicamp – Farmacologia

Sepsis is a systemic inflammatory disease with a mortality rate above 50% and sepsis by gram-positive bacteria has a higher mortality rate when compared with sepsis by gram-negative bacteria. However, experimental models that adequately mimic the signs of sepsis by gram-positive bacteria are limited. An inefficient neutrophils (NE) mobilization in *Staphylococcus aureus*-induced sepsis is the main cause of death in patients infected with this bacterium. *Staphylococcus aureus* pathological effects are strong related to the secretion of staphylococcal enterotoxins (SEs). Recently, we have shown that human eosinophils and mice bone marrow granulocytes incubated with SEs exhibit reduced *in vitro* chemotaxis and adhesion activity. The present study **Aims:** to identify the effects of SEA and SEB on the functional properties of human NE as well as the relevance of MHC class II activation for their effects. Blood was collected from healthy volunteers after approval from the local ethics committee (Protocol No 61370616.3.0000.5412). Blood samples were placed on isotonic Percoll solution. After centrifugation, the mononuclear cell layer was removed and the pellet containing erythrocytes and granulocytes was aspirated and subjected to isotonic lysis. After lysis cells (98% NE) were resuspended to 4×10^6 cells/ml and submitted to *in vitro* incubation with SEA or SEB (100 ng/ml; 2 h). Adhesion assays were carried out in 96-well plates pre-coated with recombinant human VCAM-1 and ICAM-1 for 30 min in the presence of interleukin-8 (IL-8). The NE adhesion was calculated by measuring myeloperoxidase (MPO) activity on adherent cells. NE migration assay was performed using a 48-well microchemotaxis Boyden chamber. In separated assays, neutrophils were incubated for 30 min at 37ºC with human anti MHC class II blocking antibody (2 µg/ml) before addition of SEA, SEB or MEM. Both SEA and SEB reduces human neutrophil adhesion in VCAM-1 (Control: 4.7 ± 0.5; untreated NE + IL-8: 6.7 ± 0.6; SEA treated NE+IL-8: 4.9 ± 0.8; SEB treated NE + IL-8: 3.7 ± 0.6*# optic density/NE $\times 10^6$ cells;) or ICAM-1(Control: 4.3 ± 0.5; untreated NE + IL-8: 6.1 ± 0.6; SEA treated NE+IL-8: 5.5 ± 1.0; SEB treated NE + IL-8: 3.7 ± 0.9 optic density/NE $\times 10^6$ cells) coated plates. Similar results were observed for NE chemotactic activity (Control: 18.7 ± 0.8; untreated NE +IL-8: 52.9 ± 2.7; SEA treated NE+IL-8: 28.5 ± 1.4; SEB treated NE + IL-8: 34.2 ± 1.4 NE per high-power field). Anti-MHC class II antibody prevented the reductions by SEA or SEB on IL-8-induced neutrophil adhesion and chemotaxis in comparison to non-treated cells. The inhibitory effect of SEA and SEB on human NE *in vitro* adhesion and chemotaxis via MHC class II activation suggests a role of these toxins on the neutrophil dysfunction associated with the severe sepsis by *Staphylococcus aureus*. **License number of ethics committee**: 61370616.3.0000.5412 **Financial support**: Capes

04.045 Methyl gallate attenuates Toll-Like ligands-induced inflammation: **effect on NF- κ B and MAPK activation**. Correa LB[1], Seito LN[1], Cunha TM[2], Rosas EC[1], Henriques MG[1] - [1]Fiocruz – Farmacologia Aplicada, [2]FMRP-USP – Farmacologia

Introduction: Methyl gallate (MG) is a prevalent phenolic acid in the Plant Kingdom, and its presence in herbal medicines might be related to its remarkable biological effects, such as antioxidant, antitumor and antimicrobial activities. Recently, we demonstrated that MG (0.7-70 mg/kg) inhibited zymosan-induced experimental arthritis in a dose-dependent manner. The oral administration of MG (7 mg/kg) attenuated arthritis induced by zymosan, affecting edema formation, leukocyte migration and the production of inflammatory mediators (IL-1β, IL-6, TNF-α, CXCL-1, LTB_4 and PGE_2). Zymosan is recognized through Toll-like receptor (TLR)-2 and dectin-1 receptor, resulting in the activation of reactive oxygen and nitrogen species, such as the nitric oxide (NO), NF-κB and MAPKs thereby triggering the production of inflammatory cytokines and chemokines. Herein, we investigated the MG effect in macrophage stimulated with Toll-like receptors agonists to advance knowledge about its mechanism of action. **Methods:** To assess the effect of MG (0.1-100 µM) on macrophage activation, we evaluated the production of nitric oxide, TNF-α, IL-6 and CXCL-1/KC release induced by zymosan, LPS, Pam_3CSK_4 or PMA on RAW 264.7 cell line *in vitro*. Viability was also evaluated on macrophage by MTT assay. To investigate action mechanism, RAW264.7 macrophages with a luciferase reporter gene controlled by the NF-κB promoter were used. Additionally, MG effects upon the transcriptional factors NF-κB, p-ERK1/2 and p-JNK were evaluated by western blotting in cells activated with specific TLR-2 agonist (Pam_3CSK_4). **Results:** Pretreatment with MG impaired *in vitro* production or release of cytokines TNF-α, IL-6 and CXCL-1/KC by zymosan-, LPS-, and Pam_3CSK_4-stimulated macrophages. Regarding the possible molecular mechanism of action, RAW264.7 cells stably expressing the NF-κB-luciferase reporter gene was used. MG reduced the luminescence emission by RAW264.7-Luc stimulated by different compounds but not by PMA. Moreover, pretreatment with MG reduced the phosphorylation of proteins ERK-1/2 and JNK MAPK, decrease IκB degradation and nuclear translocation of p65 NF-κB subunit induced by Pam_3CSK_4 on macrophages. **Conclusion:** The results of this study suggest that MG inhibits the activation of macrophages stimulated with TLRs agonists, by reducing MAPKs activation and NF-κB activation and translocation, which leads a decrease in the production of cytokines. **Financial support**: FAPERJ, CNPQ, CAPES

04.047 Quercetin accelerates resolution of lung pathological changes caused by silica particles in mice. Guimarães FV[1], Ferreira TPT[1], Arantes ACS[1], Jannini-Sá YAP[1], Oliveira TAL[1], Silva CD[1], Moraes JA[2], Martins MA[1], Silva PMR[1] [1]Fiocruz – Inflamação, [2]UFRJ – Labio RedOx

Introduction: Silicosis is a chronic, potentially fatal and irreversible lung disease caused by inhalation of silica particles, characterized by fibrosis and granuloma formation. To date, no effective therapy is available for treatment of silicosis. Quercetin is a flavonoid present in several plants including fruits, vegetables and some grains, known by its antioxidant and anti-inflammatory activities. This study was undertaken in order to investigate the therapeutic effects of quercetin on lung inflammatory and fibrogenic components in response to silica particles in mice. **Methods:** Male Swiss-Webster mice were anesthetized with halothane, and then instilled with crystalline silica (10 mg; particle size 0.5 – 10 µm) or saline (25 µL) by intranasal via. Treatment with quercetin (2.5 - 10 mg/kg, po.) was performed daily, from day 21 to 27. The analyses were performed 1 day after the last drug administration. Parameters included *in vivo* and *in vitro* systems, addressing oxidative stress as well as inflammatory and fibrotic markers. All experimental procedures were performed according to the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (LW57/14). **Results:** We found that therapeutic treatment of silica-challenged mice, with the flavonoid quercetin, reduced the generation of reactive oxygen species (ROS), nitric oxide (NO), peroxynitrite ($NO3^-$) in the lungs. Also, markers of oxidative damage such as elevation of malondialdehyde (MDA), 8-isoprostane and nitrotyrosin were also suppressed by quercetin. Moreover, although reduction of catalase (CAT) activity was not sensitive to quercetin, other enzymes including superoxide dismutase (SOD), glutathione peroxidase (GPx), NADPH oxidase 4 (NOX-4) and nitric oxide synthase (iNOS) had their basal levels restored by the flavonoid. Up-regulation of TNF-α, IL-1 β, IL-6, TGF-β and chemokines, as well as increased collagen deposition and airway hyper-reactivity to methacholine were all clearly sensitive to quercetin. Interestingly, we detected, for the first time, increased numbers of microvesicles in the bronchoalveolar lavage from silicotic mice, a response sensitive to quercetin, indicating that microvesicles may be considered as modulators and novel targets of silicosis. In another set of experiments, we showed that quercetin also inhibited MCP-1 generation from cultured lung fibroblasts triggered by IL-13 as well as by supernatant from silica-stimulated alveolar macrophages. **Conclusion:** Altogether, our findings show that therapeutic treatment with the flavonoid quercetin reversed important pathological features of silica particle inhalation, suggesting that it seems to be a promising compound for future application in chronic lung diseases such as silicosis. **License number of ethics committee**: Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (LW57/14) **Financial support**: FIOCRUZ, CNPq, FAPERJ, CAPES (Brazil)

04.049 Piracetam inhibits UVB-induced skin inflammation in hairless mice. Sepulveda JD[1], Rossaneis AC[2], Pinho-Ribeiro FA[2], Medeiros DC[2], Vale DL[1], Baracat MM[1], Georgetti SR[1], Verri WA[2], Casagrande R[1], Martinez RM[1] [1]UEL – Ciências Farmacêuticas, [2]UEL – Ciências Patológicas

Introduction: Skin damage caused by UVB radiation is potentiated by the following inflammatory response and inhibition of inflammatory pathways associated with neutrophil recruitment and activation can highly suppress its deleterious effects (FUJIMURA et al., 2016). Piracetam is a prototype of nootropic drugs used to improve cognitive impairment. However, recent studies suggest that piracetam can have anti-inflammatory effects (Navarro et al., 2013). Herein, we analyze whether piracetam has anti-inflammatory effects in model of skin inflammation induced by UVB radiation. **Methods:** Hairless mice were divided in five groups (n=5): non-irradiated control, irradiated control (4.14 J/cm^2) and three group of irradiated and treated with piracetam (30-300 mg/kg, via oral) 1 h before the irradiation and 8 h after the first dose. The UVB source used was a Philips TL/12 RS 40W with a peak emission at 313 nm. Mice were terminally anesthetized at 4h (interleukin 33) or 12h (other tests) after the end of the irradiation. The skin edema was measured as an increase in dorsal skin weight. The UVB-induced neutrophil migration was evaluated by myeloperoxidase (MPO) activity assay. Sodium dodecyl sulphate polyacrylamide gel electrophoresis substrate-embedded zymography was used to detect enzymes with gelatinase activity. The IL-33 level was determined by an enzyme-linked immunosorbent assay (FUJIMURA et al., 2016). Data were statistically analyzed by one-way ANOVA followed by Tukey's test, $p<0.05$. The Animal Ethics Committee (CEUA process 8909.2015.89) of the Londrina State University approved all procedures of this study. **Results:** The UVB irradiation-induced skin edema was significantly inhibited in a dose-dependent manner by treatment with piracetam, the high-dose (300 mg/kg), resulted in a significant inhibition of edema. UVB irradiation also elevated MPO activity, and this increase was inhibited by the doses of 30-300 mg/kg. Considering that MMP-9 may be produced by neutrophils and neutrophil-derived mediators may induce activation and overexpression of MMP-9, we investigated the effects of piracetam treatment in MMP-9 activity. It was observed that UVB irradiation induced a significant increase of MMP-9 activity in the skin, which was inhibited by piracetam (300 mg/kg). Furthermore, UVB irradiation induces the production of pro-inflammatory cytokine IL-33, which was inhibited by the dose of 300 mg/kg of piracetam. **Conclusion:** The anti-inflammatory mechanisms of piracetam were related to inhibition of production of IL-33 as well as reduction of MMP-9 activity. These results demonstrate that piracetam presents anti-inflammatory effect by a mechanism dependent on inhibition of cytokine production. Considering its safety and clinical use for cognitive function, it is possible that piracetam represents a novel perspective to reduce UVB irradiation-induced skin damages and merits further studies. **Acknowledgments:** CAPES, CNPq, Fundação Araucária and UEL. **References:** Fujimura, AT, J. Nat. Prod., 27, 1329, 2016. Navarro, SA, Pharmacol. Biochem. Behav., 105, 183, 2013. **License number of ethics committee**: 8909.2015.89 **Financial support**: CAPES, CNPq, Fundação Araucária and UEL

04.050 Piracetam increases Interleukin 10 production and inhibits oxidative stress induced by ultraviolet B radiation. Rossaneis AC[1], Pinho-Ribeiro FA[1], Medeiros DC[1], Baracat MM[2], Georgetti SR[2], Verri WA[1], Casagrande R[2], Martinez RM[2], Sagae BN[2] [1]UEL – Ciências Patológicas, [2]UEL – Ciências Farmacêuticas

Introduction: UVB irradiation may cause oxidative stress-dependent skin cancer and premature aging (Martinez et al., 2018). Piracetam, a derivative of the neurotransmitter gamma-aminobutyric acid (GABA), is useful in the treatment of cognitive disorders and dementia. However, recent studies suggest that piracetam can inhibits oxidative stress and inflammation in animal moldes (Navarro et al., 2013). Thus, the present study aimed to investigate the efficacy of per oral administration of piracetam in UVB irradiation-induced oxidative damage in the skin of hairless mice and the underlying mechanisms. **Methods:** Hairless mice were divided in five groups (n=5): non-irradiated control, irradiated control (4.14 J/cm^2) and three group of irradiated and treated with piracetam (30-300 mg/kg, via oral) 1 h before the irradiation and 8 h after the first dose. The UVB source used was a Philips TL/12 RS 40W with a peak emission at 313 nm. Mice were terminally anesthetized with 5% isoflurane 12 h [for 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging ability assay, and levels of endogenous antioxidant reduced glutathione (GSH)], 2 h (for superoxide anion production test) or 4 h [for lipid hydroperoxides (LOOH) assay, and levels of interleukin 10 (IL-10) test] after the UVB exposure. Afterward, the full thickness of the dorsal skins was removed and used to each test. The ABTS radical scavenging ability was measured by the decrease of absorbance at 730 nm. The GSH levels were determined by the 5,5'dithiobis (2 nitrobenzoic acid) (DTNB) assay. The LOOH production was determined by tert-butyl hydroperoxide-initiated chemiluminescence assay. The measurement of superoxide anion production was performed using the nitroblue tetrazolium assay. Furthermore, the cytokine level was determined by an enzyme-linked immunosorbent assay (Martinez et al., 2018). Data were statistically analyzed by one-way ANOVA followed by Tukey's test, $p<0.05$. The Animal Ethics Committee (CEUA process 8909.2015.89) of the Londrina State University approved all procedures of this study. **Results:** Treatment with piracetam in a dose-dependent manner significantly inhibited (300 mg/kg) antioxidant depletion (ABTS and GSH tests), maintaining the antioxidant capacity similar to control group (non-irradiated). UVB irradiation induced an increase in LOOH and superoxide anion production, and this increase was inhibited by the dose of 300 mg/kg of piracetam. Moreover, piracetam treatment increased IL-10 levels in the skin after UVB exposure. **Conclusion:** The beneficial effectiveness of piracetam included targeting production of IL-10 and also oxidative stress. Thus, these data suggest the administration of piracetam as promising therapeutic approach in skin photodamage and other inflammatory diseases where excessive reactive oxygen species production are involved. Furthermore, considering its safety and clinical use for cognitive function, piracetam merits further studies. **Acknowledgments:** CAPES, CNPq, Fundação Araucária and UEL. **References:** Martinez, RM, J Dermatol Sci., 18, 30201, 2018. Navarro, SA, Pharmacol. Biochem. Behav., 105, 183, 2013. **License number of ethics committee**: 8909.2015.89 **Financial support**: CAPES, CNPq, Fundação Araucária and UEL.

04.051 Evaluation of anti-inflammatory and antiproliferative activity of gold nanoparticles. de Paula T, Ferreira JCP, Pawloski PL, Soley BS, Ferreira GK, Ventura ACSSB, Otuki MF, Cabrini DA UFPR – Farmacologia

Introduction: Gold nanoparticles (AuNPs) are investigated for a wide variety of biomedical applications due to their biocompatibility, easiness for conjugation with biomolecules and therapeutic properties in inflammatory processes. However, this anti-inflammatory activity has not yet been investigated in skin inflammation models. Therefore, this study **Aims:** to evaluate the anti-inflammatory and antiproliferative activity of gold nanoparticles in an animal model of chronic skin inflammation and *in vitro* analysis of keratinocytes. **Methods:** The AuNPs used had a medium diameter of 10 nm, spherical shape and were stable in a colloidal dispersion. Swiss mice underwent chronic inflammatory process induction by topical application of TPA (2.5 µg/ear) on alternate days, for nine days. Animals were divided in groups: naive, TPA, AuNPs 0.1%, sodium citrate 0,008% (vehicle equivalent of AuNPs 0.1%), AuNPs 0.3%, sodium citrate 0.024% (vehicle equivalent of AuNPs 0.3%), nonionic cream (vehicle) and dexamethasone 0.1% in nonionic cream (positive control). Treatments were prepared with nonionic cream and applied topically, twice a day, from the fifth day of the trial up to the ninth day. Ear thickness was measured every day and on the ninth day of the experiment, animals were euthanized and biopsies of ear tissue were collected and submitted to analysis of the activity of the Mieloperoxidase (MPO) and N-acetyl-β-D-glucosaminidase (NAG) and histological analysis. All experiments were approved by Animal Research Ethical Committee. Human keratinocytes (HaCaT cell line) were treated with AuNPs diluted in sodium citrate in concentrations of 1, 3, 10 and 30 mg/mL and with as dispersions of sodium citrate, equivalents to the AuNPs tested concentrations. MTT and Neutral red assays were accomplished in 24 h and 72 h for the cell proliferation MTT assay. **Results:** Although the repeated application of TPA caused similar changes to psoriasis in the ears of the mice (edema, cellularity, increase in epidermis diameter, MPO and NAG) when compared to control, topical treatment with AuNPs caused no statistical difference in ear edema, histology, MPO and NAG enzyme activity when compared to control and vehicle groups (sodium citrate and nonionic cream). As for the *in vitro* results, AuNPs did not alter cell viability at concentrations of 0.3 to 10 mg/mL, but reduced viability in 43.25% ± 15.7% at the concentration of 30 mg/mL on MTT assay. In the neutral red assay, sodium citrate (AuNPs vehicle) decreasedviability at concentrations of 10 and 30 mg/mL in 40.79% ± 10.6% and 53.81% ± 10.6%, respectively. Considering the effect of the vehicle, AuNPs did not interfere with cell viability. Similar interference of the vehicle (sodium citrate) occurred with proliferation of keratinocytes after 72 h incubation, with inhibition of 82.5% ± 15.3%. **Conclusion:** The AuNPs were not able to promote efficacy in inflammatory parameters when applied topically to the inflamed skin of mice. Likewise, the *in vitro* assays showed that concentrations of AuNPS tested did not alter cell viability and proliferation in HaCaT cell. Therefore, the reducing agent used (sodium citrate) alter the results obtained, and possibly the use of other reducing agents and other sizes of nanoparticles in new tests could result in different data. Acknowledgements: INCT, CNPq and CAPES. **License number of ethics committee**: 1045

04.052 Suppression by ipatropium bromide of late lung fibrosis caused by silica particles in mice. Correa AMC, Ferreira TPT, Arantes ACS, Lima JCS, Silva CD, Martins MA, Silva PMR Fiocruz – Fisiologia e Farmacodinâmica

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. Ipatropium bromide (Atrovent ®) is a medication know to induce dilatation of medium and large airways and frequently used in the case of pulmonary diseases such as COPD and asthma. This study was undertaken to investigate the potential effect of ipratropium bromide on inflammation and fibrosis caused by silica particles instillation. **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 i) lung tissue morphology/morphometry and ii) lung function by invasive plethysmography. Animals were administered by aerosolization daily with the compound ipratropium (0.5 mg/mL) 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 observed that silicotic mice exhibited a time-dependent leukocyte infiltration in the lung parenchyma, together with collagen deposition and granuloma formation during the course of the disease. An increase in the basal levels of lung resistance and elastance, as well as airways hyper-reactivity to methacholine aerosolization was also noted. Under condition of therapeutic treatment of silicotic mice with ipratropium bromide led to restoration of lung function, including resistance and elastance, and also airways hyper-reactivity to methacholine. Ipratropium bromide also suppressed tissue fibrosis and granuloma formation. Values of granuloma area decreased from 21.7% ± 3.9% (mean ± SEM; n= 8) to 9.4% ± 2.6 % ($p<0.05$) in non-treated and ipratropium bromide-treated silicotic mice, respectively. **Conclusion:** Altogether, our findings show that mice responded with lung function decrease and fibrosis, when challenge with silica intranasally. Treatment with ipratropium bromide suppressed these phenomena, suggesting that it may be considered as a promising therapeutic tool to be used in the case of lung fibrotic diseases such as silicosis. Additional experiments are need in order to clarify better the mechanism of action of ipratropium bromide. **License number of ethics committee**: LW57/14 **Financial support**: FIOCRUZ, CNPq and FAPERJ.

04.053 Effects of treatment with a H_2S-releasing prednisone derivative on airway remodeling in a murine model of asthma. Colombo FF[1], Villela Filho GJM[1], Severino B[2], Santagada V[2], Caliendo G[2], Costa SKP[3], Muscará MN[3], Ferreira HHA[1] [1]São Leopoldo Mandic –Inflammation, [2]Università degli Studi di Napoli "Federico II" – Pharmacy, [3]ICB-USP – Pharmacology

Introduction: Allergic asthma is a complex and chronic inflammatory disorder associated with airway hyper-responsiveness and tissue remodelling of the airway structure. A growing number of observations suggest that endogenous hydrogen sulfide (H_2S) might be of biological relevance in the pathogenesis of airway diseases, such as asthma. Current asthma treatment strategies primarily involve inhaled bronchodilators and inhaled steroids, which provide significant control for mild-to-moderate asthmatics. Oral corticosteroids (e.g. prednisolone) are limited to severe or difficult-to-control asthma due to the risk of side effects from chronic systemic exposure. In this study, we compared the effects of prednisone with those from a prednisone-H_2S derivative. **Methods:** All experiments were approved by the Animal Ethics Comitee/SLMandic (license n. 2017/033). Balb/c mice were sensitized with subcutaneous injections of a solution containing 100 µg ovalbumin adsorbed in 4 mg of aluminum and potassium hydroxide on days 1 and 12. On day 12 the mice also received intranasal administration of OVA (100 µg in 25 µl saline). After sensitization, the mice were submitted to the protocol of chronic exposure to the allergen, with intranasal administration of OVA every 2 days, with an interval of 13 days and the animals were then left to recover for 4 weeks. During the recovery period a group of OVA-challenged mice received Prednisone (5,5 mg/kg; p.o.) while another group was treated with an equimolar dose of Prednisone-H_2S (9,1 mg/kg). After euthanasia, the lungs were obtained, and histological sections were submitted to different staining procedures in order to evaluate peribronchiolar extra cellular collagen (Masson Tricomium), smooth muscle actin thickness (immunohistochemistry to smooth muscle actin) and eosinophils infiltration (Sirius Red). All analyzes were performed using an optical microscope connected to an image analysis system (ImageJ). **Results:** Our analyses show that treatment with Prednisone-H_2S is significantly ($p<0.05$) more effective in reducing % of peribronchiolar collagen density (3.2□0.2), smooth muscle actin thickness (18.6□0.6 µm²) and eosinophil (0.6□0.08/field) infiltration than Prednisone alone (%collagen density: 6.0□0.4; SMA: 26□0.4 µm²; eosinophils: 1.1□0.05/field) as compared to asthmatic non-treated mice (%collagen: 24.7□1.0; SMA: 53.8□1.9µm²; eosinophils: 4.5□0.1/field). **Conclusion:** The present study shows that by the protective effect on airway remodeling, Prednisone-H_2S could be a therapeutic target for alleviating the symptoms of allergic asthma. **License number of ethics committee**: 2017/033

04.054 Changes in the mRNA expression of TLR4 and TLR2/TLR6 signaling elements in response to lipopolysaccharide and lipotheicoic acid in the mouse epididymis. Almeida PGC[1], Andrade AD[1], Kushima H[1], Avellar MCW[2], Silva EJR[1] - [1]Unesp – Farmacologia, [2]Unifesp-EPM – Farmacologia

Introduction: The epididymis plays crucial roles in sperm maturation, storage and protection. Inflammation of the epididymis, known as epididymitis, affects its functions and may result in infertility. Bacterial infections are the most common cause of epididymitis. The epididymis expresses different members of the Toll-like receptor family, such as TLR4 and TLR2/TLR6, which bind lipopolysaccharide (LPS) and lipotheicoic acid (LTA) from Gram-negative and Gram-positive bacteria, respectively, triggering innate immune responses. LPS interacts with TLR4 homodimer and its accessory proteins CD14 and MD2, activating MyD88- and TRIF-dependent signalling pathways. LTA interacts with TLR2/TLR6 heterodimer recruting acessory proteins CD14 and CD36 resulting in the activation of MyD88-dependent pathway only. We previously demonstrated that activation of TLR4 by LPS and TLR2/TLR6 by LTA in the rat epididymis recruited distinct sets of inflammatory mediators. Here, we investigated the effects of LPS- and LTA-induced acute epididymitis on the expression profile of genes involved in TLR4 and TLR2/TLR6 signaling pathways in the mouse epididymis. **Methods:** Male C57BL/6 mice (90 days, n=4-7/group) were anesthetized with ketamine/xylazin (60/120 mg/kg, ip). The vas deferens was exposed by a scrotal incision and 10 µl of sterile saline (0.9% NaCl) containing or not ultrapure LPS from *E. coli* (50 µg) or LTA from *S. aureus* (125 µg) were retrogradly injected into its lumen. Mice were killed 6 and 24 h after treatment and their epididymides were collected and processed for total RNA extraction, cDNA synthesis and quantitative polymerase chain reaction (qPCR) for evaluation of *Tlr2*, *Tlr4*, *Tlr6*, *Cd14*, *Cd36*, *Myd88*, *Trif* and *Hprt1* (endogenous control). A group of untreated mice was also killed and their testis, epididymis, vas deferens, seminal vesicles and prostate were processed as described above for conventional PCR analysis. Results were expressed as mean ± SEM and analyzed by ANOVA followed by Bonferroni test ($p<0.05$ was considered significant). **Results:** We observed the expression of *Tlr2*, *Tlr4*, *Tlr6*, *Cd14*, *Cd36*, *Myd88* and *Trif* in all reproductive organs of untreated mice, except for the abscence of *Tlr6* and *Cd36* in the prostate. The relative expression of *Tlr4* and *Tlr6* in the cauda epididymis was not changed in LPS- and LTA-treated mice in comparison to saline-control. LPS treatment increased the expression levels of *Tlr2* at 6 and 24 h (fold-change(FC) ~10 and ~3, respectively). LPS also increased the expression of *Myd88* (FC ~2) and *Cd14* (FC ~3) at 6 and 24 h, respectively, and decreased the expression of *Cd36* (FC ~-3) at 24 h in comparison to saline-control. LTA, on the other hand, increased the expression of *Tlr2* (FC ~3) and *Trif* (FC ~2) at 6 h only. **Conclusions:** We demonstrated that the recruitment of TLR4 and TLR2/TLR6 signaling pathways by luminal LPS and LTA resulted in the differential expression of transcripts enconding these receptors and their accessory and adaptor molecules in the epididymis. Our results shed new light into the epididymal acute inflammatory events in response to bacterial-derived products. **Support:** FAPESP (2017/25982-1 and 2015/08227-0). **Ethics Committee approval**: 1069-CEUA. **License number of ethics committee**: 1069-CEUA **Financial support**: FAPESP (2017/25982-1) e FAPESP (2015/08227-0)

04.055 Assessment of anti-inflammatory activity of new inhibitor of $mPGES_1$ in arthritis model in mice. Gomes RO, Santana-Junior JCV, Fróes TQ, Castilho MS, Soares DM UFBA – Farmácia

Introduction: Rheumatoid arthritis (RA) is an autoimmune pathology of progressive inflammatory trait, in which genetic and environmental factors contribute to the loss of tolerance to its own antigens. This pathology affects about 0,5-1,0% of the world's adult population[1]. The known undesirable gastric and cardiovascular effects of analgesics, and the toxic effects of immunosuppressant and monoclonal antibodies, play a key-role for RA treatment poor-adherence[2].Consequently, the development of novel drugs to treat inflammatory diseases is an unsolved problem that requires further investigation. The mPGES1 enzyme has been considered as a promising target to achieve this goal[3]. **Objectives:** The objective of this research is to assess the anti-inflammatory activity of a putative $mPGES_1$inhibitor (Z1390) on a zimosan-induced arthritis model, using mice. **Materials and Methods:** Male mice from Swiss lineage were employed for all experiments, as approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine of UFBA, under protocol n°. 58/2017**.** The putative MPGES1 inhibitor (Z1390), which has been identified by virtual screening protocol[4] and acquired from ENAMINE, was solubilized in PluronicF68®. The animals were submitted to treatment before and after induction of arthritis with zimozan (intra-articular injection). The swelling of the knee joint was assessed by measuring the transverse diameter of the left knee using a digital caliper, two, four and six hours after zymosan injection. The leukocyte migratory profile was evaluated by cellular lavage with couting in the Neubauer chamber. In the experimental standardization, the increase of the proliferative profile of neutrophils and joint diameter was verified in the animals submitted to zymosan. The data were analyzed by two-way ANOVA and considered significant when $P< 0.05$. **Results:** Once pluronic F-68 does not influence the inflammatory response, it is a suitable vehicle for i.v. administration of drugs5[4]. The leukocyte count and joint diameter measurement showed that Z1390, at 1mg/kg dose, is as potent as dexamethasone, at 2mg /kg dose. At this dose, it was observed to lower leukocyte proliferation and joint diameter variation, in comparison to either to saline/zymozan or PluronicF68®/zymozan treat groups ($p <0.05$).**Conclusion:** These preliminary results suggest that the Z1390 is a promising lead-compound for the development of novel anti-inflammatory drugs, with reduced incidence of adverse effects, that shall improve the arsenal of drugs for joint inflammation treatment. However, further studies are required to support this claim.

04.056 Systemic infection was increased in low-birth-weight rats induced by intrauterine malnutrition. Azevedo GA, Gil NL, Balbino AM, Rodrigues LGA, Carvalho MM, Silva MM, Landgraf MA, Landgraf RG Unifesp-Diadema – Ciências Farmacêuticas

Metabolic disorders can also be caused by the imbalance of pro and anti-inflammatory responses in the body, similar to what is observed in the establishment of sepsis. In previous studies, our group demonstrated global intrauterine malnutrition resulted in low birth weight, hypocellularity in bone marrow and peripheral blood, reduction in leukocyte migration and decreased of the inflammatory mediators in Wistar rats. In this same model, we also observed reduction of acute and allergic pulmonary inflammation, decreased cytokines production and high levels of circulating corticosterone. The aim of this study was to correlate if the lower inflammatory response observed in malnourished rats could mean a higher propensity to develop infections in these animals. Females Wistar rats in estrus were mated and, after confirmed the presence of spermatozoa in vaginal swab, were divided into two groups: G1 - fed with normal diet; G2- 50% food restriction. Offspring G1 of normal birth weight (NBW) and offspring G2 of low birth weight (LBW). Six hours after sham operation or cecal ligation and puncture (CLP) in offspring male rats these rats were euthanasied. We observed hypothermia and hyperglycemia in animals with sepsis, and the glycemia of NWB with sepsis was higher than that of the LBW with sepsis group. Cytokines and the hormone Leptin were evaluated in serum and lung tissue by Multiplex. Both serum and lung tissue have shown that leptin increased during sepsis, as well as the cytokines IL-1b, IL-10, IL-6, IL-8. These cytokines are higher in the NWB with sepsis than in the LBW with sepsis group. Bronchoalveolar lavage fluid showed a reduction in the cellular infiltrate (mononuclear cells) in both groups with sepsis. The peritoneal lavage presented an increase in the total cells number in NBW and LBW groups. Differential cell analysis showed that this increase was represented by polymorphonuclear cells (neutrophils). Our preliminary results indicate that low birth weight rats with induced by intrauterine malnutrition are larger predisposed to systemic infection such as sepsis than animals of normal birth weight.

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04.057 The effect of cannabinoids in the reduction of inflammatory response in graft-versus-host-disease in mice. Berg BB[1], Soares JS[1], Paiva IR[1], Silva VP[2], Teixeira MM[2], Rezende BM[3], Campos AC[4], Rachid MA[5], Romero TRL[6], Romero MGMC[6] - [1]ICB-UFMG – Farmacologia e Fisiologia, [2]ICB-UFMG – Morfologia, [3]UFMG – Enfermagem, [4]FMRP-USP – Farmacologia, [5]ICB-UFMG – Patologia, [6]ICB-UFMG – Farmacologia

Introduction: Graft-versus-host-disease (GVHD) is an illness secondary to allogeneic bone marrow transplant, leading to a generalized inflammation in host, causing damage to several organs. Therefore cannabinoids, know to modulate inflammatory response, such as endocannabinoids like Anandamide (AEA) and Palmitoylethanolamide (PEA), and the phytocannabinoid Cannabidiol were assessed upon their effect on GVHD. **Objectives:** This study evaluates the effect of cannabinoids concerning the inflammatory response of mice submitted to experimental GVHD. **Methods:** GVHD was induced in Balb/c mice by the transplant of $1x10^7$ bone marrow cells and $1x10^7$ splenocytes from C57BL/6 mice. GVHD-mice were treated, every 24h, with intraperitoneal injections containing AEA (10 mg/kg), PEA (10 mg/kg), CBD (10, 30 and 60mg/kg) and the vehicle of each group, from the day of disease induction continuing for 6 days until euthanasia. After transplant, mortality was assessed daily. To evaluate survival, mice were followed for 25 days. Inflammatory response was evaluated in the 6^{th} day after GVHD induction in the jejunum-ileum (JI) and liver. Histopathological analysis, quantification of cytokines/chemokines (ELISA) and flow cytometry were performed. **Results:** The endocanabinoids PEA and AEA increased survival respectively by 83% and 66% and CBD 30 mg/Kg was the most effective dose with 83% survival rate, and therefore became our dosage of choice for further treatments. When injury reduction in the jejunum-ileum and liver was assessed CBD and PEA presented the smaller tissue damage, meaning an improved histopathological score; even though AEA also showed to have some degree of protectiveness. CBD reduced TNF-α, IFN-γ, CCL2 and CCL3 in JI and CCL3 and TNF-α in liver. As for the endocanabinoids, PEA did not reduce the levels of any cytokines or chemokines evaluated and AEA reduced only TNF-α in the liver and neither had meaningful effect in JI. Conversely, flow cytometry of liver, spleen and JI revealed that both PEA and AEA treatment resulted in the reduction in the number of $CD4^+$ and $CD8^+$ lymphocytes, lymphocyte activation by stained $CD28^+$. CBD displayed a very different profile from the endocanabinoids, with no significant change in the spleen and the increase of all analyzed lymphocytes in liver and JI. Prominencially $CD4^+FoxP3^+$ population, known as T-Helper lymphocyte, was increased in the CBD group. These results elucidate why CBD did not reduce GVHD's anti-tumoral effect (GVL), but still protects the host against GVHD inflammation. **Conclusion:** CBD treatment reduced mortality of mice by decreasing inflammation, injury and promoting regulation in jejunum-ileum and liver. PEA and AEA also reduced mortality by reducing the inflammatory infiltrate. These findings suggest that both endocanabinoids and cannabidiol have potential as an alternative or complementary treatment for GVHD. **License number of ethics committee**: 337/2014 **Financial support**: Capes, CNPq, PRPQ, FAPEMIG

04.058 Cardiac inflammation in mice Graft-versus-Host Disease. Paiva IR[1], Berg BB[1], Soares JS[1], Rachid MA[2], Cau SB[3], Castor MGM[3] [1]ICB-UFMG – Farmacologia e Fisiologia, [2]ICB-UFMG – Patologia, [3]ICB-UFMG – Farmacologia

Introduction: Hematopoietic stem cell transplant is the maily curative therapy for many hematological diseases and cancer. However, the success of this therapy is often not achieved due to the occurrence of a secondary disease denominated graft versus host disease (GVHD). GVHD is one of the most common causes of mortality after bone marrow transplants. This disease involves an immune response between transplanted lymphocytes and host tissues, occurring by immune attack of donor T cells to host cells, which differ in terms of major histocompatibility complex (MHC). The organs most commonly affected by GVHD are the liver, lungs, skin, gastrointestinal tract and lymphoid tissues. However, the involvement of the cardiovascular system has already been described in humans. Moreover, cardiac autopsy of GVH patients showed inflammatory infiltrates and high levels of inflammatory mediators such as TNF-alpha and IL-2. Therefore, it becomes necessary to study the inflammatory response of the Cardiovascular System triggered by Graft-versus-Host Disease in the murine model and its functional repercussion. **Objectives**: Evaluation of the cardiac inflammation in Graft-versus-host disease (GVHD) induced mice. **Material and Methods:** C57BL/6J male mice, 8-12 weeks old, submitted to total body irradiation (9Gy). Immediately after radiation, the mice were transplanted with $30x10^6$ of Balb-c splenocytes for the 7- and 15-days groups and $1x10^6$ splenocytes for the 40 days group. All groups also received $1x10^6$ hematopoietic stem cells. After transplantation, GVHD clinical parameters were monitored each 2 days. To evaluate disease progression, mice were euthanized 7, 15 and 40 days after GVHD induction. For the morphological study, the heart was cross-sectioned and stained on H&E or Masson's trichrome to evaluate the presence of inflammatory infiltrate and collagen, respectively. **Results:** Our preliminary results suggest that at 7, 15 and 40 days of induction the mice showed a progressive increase of inflammatory infiltrate, predominantly in the perivascular regions. At 15 and 40 days after transplant, we noticed a perivascular collagen deposition. The presence of inflammatory infiltrate in the cardiac tissue of diseased animals may be due to immune reaction of donor T cells against host cells. The increase in collagen at 15 and 40 days may contribute to a possible late cardiac dysfunction. **Conclusions**: Our preliminary findings suggest that at 7, 15 and 40 days of induction the mice showed a progressive increase of inflammatory infiltrate, predominantly in the perivascular regions. At 15 and 40 days after transplant, we noticed a perivascular collagen deposition, suggesting that the cardiac inflammation is triggered by GVHD. It’s clear the necessity of further experiments in order to elucidate the evolvement of inflammatory cells and inflammatory mediators in cardiac tissues. Moreover, we will evaluate the cardiac function of the animals **License number of ethics committee**: CEUA 337/2014 **Financial support**: Proreitoria de pesquisa (PRPQ); CAPES; CNPq; FAPEMIG

04.059 The effect of obesity on a female murine model of neutrophilic asthma
Suaiden AS[1], Oliveira MA[1], Ribeiro MR[1], Roco FPS[2], Vitorasso RL[3], Moriya HT[3], Prado CM[2], Riffo-Vasquez Y[4], Tavares WL[1] [1]USP – Farmacologia, [2]Unifesp – Biosciência, [3]USP – telecomunicação e engenharia de controle, [4]Kings Collage - Farmacologia

Rational: Clinical data indicates that obese asthmatic women are more likely to develop neutrophil-mediated asthma than men. However, there are a limited number of experimental models available for the study of the association between obesity and neutrophilic asthma. **Objectives:** Characterization of an experimental model of neutrophilic asthma in female mice and the study of the interaction between obesity and the pulmonary inflammatory phenotype. **Methods:** Female Balb / c mice were maintained on a high fat (HFD) or standard diet (SD) for 10 weeks. Mice were sensitized with ovalbumin (OVA) and Freund's complete adjuvant (CFA). Three weeks later, mice were instillated with 10 µg of OVA i.n. for 3 consecutive days. **Results:** We observed a significant increase in number of neutrophils in the lung of animals sensitized and challenged with OVA in comparison to their controls, independently of the diet (SD Sham: ND, SD OVA: 6±0.6*, HFD Sham: ND, HFD OVA: 6.3±0.2* x10^5/ml, n=10/group,* p<0.05 vs Sham). However, a significant increase in MPO activity was observed in HD mice in comparison to SD (SD OVA 2.1±0.2, HFD OVA: 3.6±0.3*.optical density (OD) 450 nm, n=5/group, *p<0.05). A significant increase in eosinophils number and EPO was observed in the lung of HFD mice in comparison to SD mice (SD OVA: 2.33±0.2, HFD OVA: 4.5±0.2*, x10^5/ml, n=10/group, *p<0.01. EPO: SD OVA: 1.17 ± 0.1, HFD OVA: 2.2±0.4*, O.D. 450 nm, n=5/group, *p<0.0001). In addition, HFD diet mice presented a significant constitutive increase in the thickness of the of airways muscle layer that remained significantly higher after sensitization (SD Sham: 8.7±0.2, SD OVA: 19.9±0.3*, HFD Sham: 18.2±0.4□, SFD OVA: 22.8±0.6*□ µm, n=10, *p<0.0001 vs Sham, □p<0.0001 vs SD Sham, □p<0.001 vs SD OVA). Similarly, we observed increment of collagen deposition around the airways (SD Sham: 2.68±0.3, SD OVA: 14.3+02*; HFD Sham: 6.6±0.05□, HFD OVA: 17.06±0.5□, % of airways area, *p<0.0001 vs Sham, □p<0.0001 vs SD Sham; □p<0.0001 vs SD OVA) **Conclusion:** Our data suggest that obesity alters the activation of leukocytes and remodeling of the airways in model of female neutrophilic asthma. **License number of ethics committee**: 914415021 5288160218 **Financial support**: FAPESP, CAPES, CNPq

04.060 Uroprotective effect of α-phellandrene in iphosphamid-induced hemorrhagic cystitis in mice. Gonçalves RLG[1], Oliveira LSA[1], Lopes ME[1], Rezende DC[1], Sousa IJO[1], Nogueira KM[2], Souza LKM[2], Medeiros JVR[2], Wong DVT[3], Pereira VMP[3], Lima-Júnior RC[3], Oliveira FA[1] [1]UFPI –Medicinal Plants, [2]UFPI – Experimental Physiopharmacology of Gastrointestinal Disorders, [3]UFC – Pharmacology of Inflammation and Cancer

Introduction: Hemorrhagic cystitis is the major dose-limiting adverse effect of the clinical use of oxazaphosphorins, including ifosfamide (IFOS). This event occurs through the formation of acrolein, a metabolite responsible for the urotoxicity of these drugs, resulting in increased oxidative stress and production of proinflammatory cytokines, culminating in tissue degradation of the bladder tissue (AL-MALKI, 2014). In previous studies, our group demonstrated that the monoterpene α-phellandrene has anti-inflammatory activity, which opens the door for its study in the attenuation of hemorrhagic cystitis. In this context, the uroprotective effect of monoterpene α-phellandrene was evaluated against the hemorrhagic cystitis model induced by ifosfamide. **Methods:** The hemorrhagic cystitis model was induced by single dose antineoplastic ifosfamide (400 mg / kg ip) preceded by pretreatment with saline and α-phellandrene (6.25, 12.5, 25, 50 and 100 mg / kg ip) in Mus musculus (CEUA 279/2016). In order to analyze the reduction of the damage, the bladder wet weight, hemoglobin content and the evans blue dye extravasation from the bladder matrix were evaluated (PUV),. In order to characterize the involvement of neutrophil migration, lipid peroxidation and involment of enzymatic and endogenous non-enzymatic antioxidants, the tissue markers Mieloperoxidase (MPO), Malondialdehyde, Nitrite / Nitrate (NOx), Superoxide dismutase (SOD) and Reduced Glutathione (GSH) were evaluated respectively. Inflammatory cytokines (TNF-α and IL-1β) were measured by ELISA immunoassay technique. Inhibition data were calculated by normalization in relation to the negative control (CN) and significance calculated considering $p < 0.05$. **Results:** The results show that pre-treatment with α-phellandrene (12.5 and 25 mg / kg) significantly reduced by 31.59 ± 0.8 and 29.90 ± 0.3%, respectively, PUV in comparison to CN. The spectrophotometric analysis showed that pretreatment significantly reduced bleeding by 65.7 ± 4% and vascular extravasation of proteins at the best dose tested (25 mg / kg) by 39.3 ± 8%. The evaluation of the tissue markers of inflammation / oxidative stress showed that in the best dose tested (25 mg / kg) α-phellandrene reduced MPO (62.13 ± 1%), MDA (25.9 ± 2%) NOx (15, 89 ± 0.1%), significantly in relation to CN ($p < 0.05$). The prevention of the depletion of endogenous antioxidants by α-phellandrene was significant and showed a percentage of 73.0 ± 18% and 86.6 ± 10% in the levels of SOD and GSH respectively. In the assessment of cytokines, α-phellandrene was able to significantly reduce TNF-α levels (28.45 ± 11.5%), but did not significantly alter IL-1β levels (22.7 ± 4%). **Conclusion:** The monoterpene α-phellandrene was able to attenuate hemorrhagic cystitis induced by ifosfamide, it showed potential inhibition of oxidative stress parameters and reduction in levels of TNF-α. **References:** AL-MALKI, A. Synergistic effect of lycopene and melatonin against the genesis of oxidative stress induced by cyclophosphamide in rats. Toxicol Ind Health, 5705, 2014 **Keywords:** Hemorrhagic cystitis; α-phellandrene; Antineoplastic chemotherapy **License number of ethics committee**: CEUA- UFFPI: 279/2016 **Financial support**: FAPEPI; CAPES; PPSUS-Ministério da Saúde

04.061 Effect of proteinase-activated receptor (PAR)2 antagonist on the neutrophil migration induced by LPS in mice C57BL/6. Almeida AD, Klein A UFMG – Farmacologia

Introduction: The Proteinase-Activated Receptors (PARs) are G protein-coupled receptors with seven transmembrane domains and activated through proteolytic cleavage at its N-terminal portion. Serine proteases released or generated during tissue injury, infection or inflammation can activate PARs-mediated cellular signaling. PAR-2 is widely distributed in the airway cells and has been associated with pulmonary inflammatory diseases. Toll-Like Receptors (TLR) are also present in the lungs and play an important role in the innate immune system, being able to recognize pathogen-associated molecular patterns. Thus, the aim of this study is investigating the importance of PAR-2 for neutrophils migration to the lung of mice induced by lipopolysaccharide (LPS). **Methods:** The experimental procedures were approved by Ethics Committee on the Use of Animals (CEUA/UFMG, 150/2017). Female C57BL/6 mice (18-20 g) were used in all experiments and housed in a temperature-controlled room with free access to food and water. The animals were pretreated by the intraperitoneal route with PAR-2 antagonist (ENMD-1068, 0.05 – 1.0 mg/kg) or serine protease inhibitor (Aprotinin,10-100 ng/30 µL) by intranasal treatment. After 1 hour they were anesthetized with Ketamine-Xylazine and treated by the intranasal route with LPS (1-100 ng/20 µL) or vehicle (PBS). After 4 hours of LPS treatment the lungs were perfused twice with 1 ml PBS to obtain the bronchoalveolar lavage (BAL). Total cells count present in BAL were performed in a Neubauer chamber using Turk's staine. Differential cell counts were performed on cytospin preparations stained with May-Grünwald solution using standard morphologic criteria to identify cell types. Bronchoalveolar lavage supernatant was used for CXCL1 cytokine analysis by the ELISA method 4 hours after the stimulus. The statistical analyzes used were One-Way ANOVA followed by the Newman-Keuls post-test. **Results:** LPS induced the migration of neutrophils into the lung (PBS 0,2525 ± 0,0465; LPS 1 ng 1,6188 ± 0,5110; LPS 10 ng 11,5958* ± 3,5482; LPS 100 ng 25,2750*** ± 4,3111 neutrophils x 10^4; $^{*}p< 0,05$ and $^{***}p< 0,001$ compared with the control group; n= 5-6). The PAR-2 antagonist significantly reduced neutrophil migration induced by LPS (PBS 0,5792 ± 0,2188; LPS 10 ng 21,7750 ± 6,5065; LPS 10 ng + ENMD 0,05 mg/Kg 4,4500*** ± 0,6551; LPS 10 ng + ENMD 0,5 mg/Kg 2,2854*** ± 0,2803; LPS 10 ng + ENMD 1,0 mg/Kg 3,7563*** ± 1,1008 neutrophils x 10^4; $^{***}p< 0,001$ compared with the LPS group; n=5-6). The serine protease inhibitor decreased neutrophil migration induced by LPS (PBS 2,9659 ± 0,5804; LPS 10 ng 87,2670 ± 8,2321; Aprotinin 10 ng/30 µL + LPS 10 ng 54,3750** ± 7,6012; Aprotinin 30 ng/30 µL + LPS 10 ng 53,5250** ± 5,3737; Aprotinin 100 ng/30 µL + LPS 10 ng 62,1675* ± 10,5271 neutrophils x 10^4; $^{*}p< 0,05$ and $^{**}p< 0,01$ compared with the LPS group; n=10-12). The ENMD-1068 decreased CXCL1 concentration 4 hours after stimulation with LPS (PBS 110,6 ± 50,52; LPS 10 ng 1137 ± 242,4; ENMD 0,5 mg/Kg + LPS 10 ng 112,8* ± 62,53 pg/mL; * p< 0,001 compared with the LPS group). **Conclusion:** PAR-2 and proteases present at the inflammatory site play an important role in the migration of neutrophils that can be mediated by the release of CXCL1 induced by LPS in lungs of mice, favoring the inflammatory response. **Financial support**: CNPq and FAPEMIG. **License number of ethics committee**: protocolo CEUA 150/2017 **Financial support**: CNPq, FAPEMIG

04.062 *In vivo* **anti-inflammatory activity of** *Ocotea minarum* **and** *Psychotria poeppigiana*. Junior PCO[1], Volobuff CRF[2], Pederiva MMC[1], Santos RC[2], Santos SM[1], Nascimento KF[2], Formagio ASN[1] [1]UFGD – Ciências Biológicas, [2]UFGD – Ciências da Saúde

Introduction: Psychotria poeppigiana Müll. Arg. known as "beijo-de-negro" and "chapéu-do-diabo", is used in the treatment of high fever, diarrhea and asthenia (Valadeau et al., 2009). Ocotea minarum (Ness & Mart.) Mez., known as "canela-vassoura", is popularly used to treat candidiasis (Rodrigues et al., 2014). The aim of this study was to evaluate the in vivo anti-inflammatory activity in paw edema and pleurisy models with the methanolic extract of P. poeppigiana (MEPP) and O. minarum (MEOM) leaves. **Methods:** The models of inflammation paw edema and pleurisy were developed with Swiss male and female mice, respectively. Doses of 30, 100 and 300 mg/kg and naive, saline and positive dexamethasone groups were evaluated (Kassuya et al., 2009). Statistical analysis was performed via ANOVA followed by Student Newman-Keuls. **Results:** The MEPP tested for paw edema model (30min, 1 hour, 2 hours and 4 hours after carrageenan-induced mice paw edema) showed inhibition percentages higher than the control at the 30min. (30 mg/kg 80,97%; 300 mg/kg 88% and Dexa 78,58%), and in 1, 2 and 4 hours, did not showed statistical difference between the positive control and the doses tested ($p>0,05$). In the pleurisy test MEPP showed a percentage of inhibition higher than the control in the dose of 100 mg/kg (93,47%; Dexa 88,84%) 4 hours after induced by carrageenan, in the pleural cavity of mice ($P < 0,05$). For MEOM there was no significant difference between the positive control and the doses of 30 and 100 mg/kg in 30min, 1 hour and 2 hours and positive control and 100mg/kg in 4 hours ($P < 0,05$). The total leukocytes migration induced by carrageenan did not present statistical difference between the positive control and the doses tested ($p>0,05$). **Conclusion:** To the best of our knowledge, this is the first evaluation of the biological activities of the plant P. poeppigiana and O. minarum, demonstrating their potential as anti-inflammatory. More tests will be necessary to prove their efficiency as possible phototherapy. . **Acknowledgments:** Capes, CNPq, Fundect and UFGD for **Financial Support.** Ethic committee process numbers: 18/2017 and 12/2017/CEUA. Valadeau et al. J. Ethnopharmacol. vol.180. p.123. 2009. Rodrigues et al. Iheringia. vol.105.p.53,2014 Kassuya et al. Rev. bras. farmacogn. vol.27. p.220. 2009 **License number of ethics committee**: 18/2017 12/2017 **Financial support**: Capes, CNPq, Fundect and UFGD

04.063 Activation of lung fibroblast by supernatant of alveolar macrophages stimulated with silica *in vitro*. Oliveira TAL[1], Correa AMC[1], Guimaraes FV[1], Ribeiro NBS[1], Sá YAJ[1], Martins MA[1], Silva PMR[1] - [1]Fiocruz – Fisiologia e Farmacodinâmica

Introduction: Silicosis is a chronic inflammatory pulmonary disease caused by the inhalation of silica particles, which has as main characteristics the formation of granuloma, excessive deposition of extracellular matrix, with subsequent loss of respiratory function. Moreover, silica particles cause direct toxicity to alveolar macrophages, leading to the release of proinflammatory mediators that promote fibroblast activation/proliferation, with subsequent excessive deposition of extracellular matrix. In this project, we investigated the cellular communication process between macrophages and fibroblasts, based on stimulation with silica particles *in vitro*. **Methodology:** Murine alveolar macrophages (AMJ2C11 line), submitted to stimulation with silica (10-300 µg/mL) or LPS (positive control) were used, and the supernatants were collected 24 h later. Analyzes included TNF-α production by ELISA and toxicity by the MTT method. Fibroblasts were obtained from the lungs of Swiss-Webster mice, normal or previously instilled with silica particles (10 mg/animal), 7 days after. After mechanical dissociation and enzymatic digestion with collagenase, the cells were maintained in culture and, after the third passage, they were characterized by cell phenotype. The analyses included proliferation by BrdU and the profibrotic chemokine MCP-1 levels in the supernatant, quantified by ELISA. Analyzes were done 24 hours after stimulation with rmIL-13 (40 ng / ml) (positive control) or with the harvested supernatant from silica-stimulated macrophages (24 h). All the procedures used were approved by the Animal Use Ethics Committee (CEUA) of FIOCRUZ (License 057/14). Results and discussion: We noted by means of MTT assay that there was no evidence of silica toxicity on the fibroblasts, at the concentrations tested (10-300 µg/mL). In another set of experiments, we observed that macrophages once stimulated with silica particles, they became activated as attested by increased levels of TNF-α detected in the supernatant. Interestingly, the supernatant recovered from silica-stimulated macrophages (24 h), when added to lung fibroblasts, induced a proliferative response and also the production of MCP-1. Values of MCP-1 generation increased from 1.083 ± 262 (pg/mL) (mean ±SEM n=5) in non-stimulated fibroblast to 2.871 ± 480 and 14.590 ± 869 in fibroblasts incubated with medium- and silica-stimulated macrophages, respectively. This response was exacerbated in the case of lung fibroblasts recovered from silicotic mice. **Conclusion:** Our results show that the supernatant from silica-stimulated macrophages induces lung fibroblast proliferation and activation *in vitro*, suggesting the existence of a communication process between these two cell types. Additional experiments are needed in order to investigate the mechanism implicated in this phenomenon. **License number of ethics committee**: License 057/14 **Financial support**: FIOCRUZ, CAPES, CNPq e FAPERJ

04.064 Effects of ovaries removal on allergic lung inflammation in obese mice. Perez Umana ER[1], Oliveira MA[1], Ribeiro RM[1], Forastieri HV[1], Riffo-Vasquez Y, Moriya HT[2], Tavares-de-Lima W[1] [1]ICB-USP – Farmacologia, [2]USP – Pharmacology

Rational: Clinical evidence shows that menopause and obesity are risk factors of worsening asthma. Experimental data regarding an interaction of these factors on asthma model still deserve studies. **Objectives:** Evaluate the influence of ovariectomy (OVx) in obese mice previously submitted to allergic lung inflammation. **Methods:** Female Balb/c mice were fed with high fat diet (HFD) for 10 weeks. Mice were sensitized with ovalbumin (OVA) + alumen and challenged 3 weeks later (OVA,10µg, intranasal, 3 consecutive days). Next, ovariectomy (OVx) was performed and the allergic mice were rechallenged 10 days later (Allergic OVx). As control were used non-manipulated obese mice (C) and obese Sham OVx allergic mice (Sham). **Results:** HFD mice increased the levels of biomarkers of obesity (glycemia, cholesterol, triglycerides, visceral fat and weight). Bronchoalveolar lavage (BAL) of rechallenged Sham mice increased the number of total cells (T) and eosinophils (Eos) relative to C group (Sham: T: 73.67 ± 13.15 vs C T: 9.92 ± 1.51, x 10^4/ml, n=9/ group, *p<0.05) and (Sham: Eos: 43.05 ± 11.24 vs C: Eos 0.022 ± 0.014, x10^4/ml, n=9/ group, *p<0.05). OVx-mice reduced uterine weight relative to Sham (OVx: 10.11 ± 1.22 vs Sham: 74.15 ± 9.19, x10^4/ml, n=10/group, *p<0.05) and its morphological analyses of vaginal smears were compatible to diestrous phase. Bronchoalveolar lavage (BAL) of rechallenged OVx mice showed higher increase the number of total cells (T), mononuclear (Mn) and eosinophils (Eos) counting relative to Sham (OVx: T: 150.02 ± 12.15 vs Sham: T: 73.67 ± 13.15, x10^4/ml, n=9/group, *p<0.05), (OVx: Mn: 36.26 ± 5.82 vs Sham: Mn: 29.71 ± 4.18, x10^4/ml, n=9/group, *p<0.05) and (OVx: Eos: 107 ± 8.15 vs Sham: Eos: 43.05 ± 11.24, x10^4/ml, n=9/group, *p<0.05). Lung mechanics of rechallenged Ovx mice showed an increased airways (Rn) and parenchyma resistance (G) to systemic administration of methacholine (MCh) as compared Sham mice (OVx: Rn: 4.54 ± 0.348 vs Sham Rn: 2.62 ± 0.285; cmH20.s/ml, n=9/group, *p<0.05) and (OVx: G: 13.01 ± 1.52 vs Sham: G: 10.24 ± 1.06, cmH20/ml, n=9/group, *p<0.05). **Conclusion:** Our data suggest that the inflammation of lung in allergic reaction, might worsen after the sexual hormones decrease and obesity may contribute to modify the magnitude of lung inflammatory phenotype. **License number of ethics committee**: CEUA: 015/2014 CEUA: 5288160218 **Financial support**: FAPESP, CAPES, CNPq

04.065 LPS-induced epididymitis differentially regulates WFDC (Whey-Acidic Protein Four-Disulfide core) gene expression in the mouse cauda epididymis.
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Introduction: Epididymitis, inflammation of the epididymis, is a prevalent cause of male infertility, affecting men's health and well-being. Bacterial infections reaching the epididymis via retrograde urethral ascension are the most common etiology of epididymitis. To fight invading pathogens, the epididymis is armed with an immune arsenal that includes the expression of antimicrobial genes, such as the members of the *WFDC* (Whey-acidic protein disulfide four core)-type protease inhibitors. Abundant expression of *WFDC* genes was detected in the human and rodent epididymis, highlighting their potential roles in immune and reproductive functions. We hypothesize that the expression of *Wfdc* genes is modified during bacterial epididymitis as part of the tissue defense mechanism against infections. Thus, our aim was to evaluate the mRNA expression of *Wfdc* genes in the epididymis of mice submitted to an experimental model of epididymitis induced by lipopolysaccharide (LPS) from *E. coli*. **Methods:** To induce epididymitis, C57BL/6 male mice (90 days, n=4-7/group) were anesthetized with ketamine/xylazin (60/20 mg/kg, ip). The vas deferens was exposed by a scrotal incision and 10 µL of sterile saline (0.9% NaCl) containing or not ultrapure LPS (12.5, 25 and 50 µg/epididymis) were retrogradly injected into the lumen of the epididymal portion of vas deferens. Mice were killed 6 and 24 h after injection and their cauda epididymis was collected and processed for total RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) studies for the evaluation of the mRNA levels of *Wfdc2*, *Wfdc4*, *Wfdc7*, *Wfdc8*, *Wfdc16*, *Il6* (interleukin-6) and *Hprt1* (hypoxanthine-guanine phosphoribosyltransferase 1, endogenous control). **Results:** Epididymis from LPS-treated mice showed macroscopic signs of edema and hyperemia at 6 and 24 h, whereas saline-control epididymis showed no signs of inflammation. The ongoing inflammation in the cauda epididymis was confirmed by an increase in the transcript levels of the inflammatory marker *Il6* 6 h after LPS challenge in comparison to saline-control (fold-change ~12, ~16, and ~17 for 12.5, 25 and 50 µg LPS, respectively; $p<0.05$, ANOVA followed by Bonferroni test). Further studies using 50 µg LPS demonstrated that LPS-induced epididymitis did not change the expression of *Wfdc4*, *Wfdc7*, *Wfdc8* and *Wfdc16* in the cauda epididymis at all time-points analyzed. The transcript levels of *Wfdc2*, however, were significantly increased in comparison to saline-control at 24 h (fold-change ~2, $p=0.02$, Student t test). **Conclusion:** Our findings demonstrate that the mouse cauda epididymis acutely increased the expression of *Wfdc2* transcript in response to an inflammatory stimulus. WFDC2 protease inhibitor and antimicrobial activities could play a role in the innate immune response of the epididymis against bacterial infections. Studies are under development to further understand how *Wfdc* genes are regulated in the inflamed epididymis, which may improve our understanding on the immunobiology of the epididymis and its impact on fertility. **License number of ethics committee**: 1029-CEUA **Financial support**: Fapesp (2017/20102-3; 2015/0227-0)