ABSTRACTS

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04. Inflammation and Immunopharmacology

04.001 TRPV1 deletion protects against cerebral malaria in mice. Pereira DMS, Murillo O, Peixoto EPM, Teixeira SA, Araújo MC, Monteiro-Neto V, Cunha TM, Marinho CRF, Muscará MN, Fernandes ES ICB-USP

Malaria is an infectious disease of global importance and presents great morbidity and mortality. Severe malaria is a neurological complication associated with brain inflammation which can be lethal or cause irreversible neuronal sequelae in surviving patients. Host´s immune response to infection plays a decisive role in the clinical evolution of malaria and therefore, influences disease outcome. The transient receptor potential vanilloid 1 (TRPV1), a Ca^{+2} permeable channel expressed on neuronal and non-neuronal cells such as endothelial and immune cells, was recently shown to be protective against bacterial infection (Fernandes et al., 2012; *J Immunol*, 188: 5741-5751). Also, this channel was pointed as a modulator of the innate immune response to malaria (Fernandes et al., 2014; *Mediators Inflamm*, 2014:506450); however, little is known of the relevance of TRPV1 in cerebral malaria. Here, we investigated the role of TRPV1 in cerebral malaria by using a mouse model of disease induced by *Plasmodium berghei* ANKA. For this, TRPV1 wild type (WT) and knockout (KO) mice received a single intraperitoneal (i.p.) injection containing *P. berghei* ANKA ($1x10^6$ infected red blood cells). Mice were observed over 14 days post-infection and the clinical evolution of disease and parasitemia were registered. Animals were then culled as soon as the disease progressed to the stages III/IV (characterized by neurological symptoms) and their brains were collected. Both WT and KO mice exhibited similar parasitemia. However, infection caused higher mortality in WT (83%) than in KO mice (17%). Whilst malaria progressed to stage III/IV in WTs, it remained at stage I in KOs. Interestingly, infected TRPV1 KO mice presented lower levels of cytokines (TNFα, IL-6 and IL-10) in their brains in comparison with those of WT infected controls. On the other hand, the cerebral levels of H_2O_2 were found to be higher in infected TRPV1KO in comparison with WT mice. Catalase activity was lower whilst glutathione reductase activity was increased in TRPV1KO brain samples in comparison with those of WT mice injected with *P. berghei* ANKA. Overall, our results show for the first time that TRPV1 plays a deleterious role in cerebral malaria, as its deletion increases survival. This was found to be due to TRPV1 ability to modulate inflammation in the brain tissue. We suggest that TRPV1 antagonists may represent a novel therapeutic approach to treat and/or prevent severe malaria. **Financial Support**: CAPES, CNPq, FAPEMA and FAPESP. Ethics Committee approval 58/2012. **Keywords**: Cerebral malaria, TRPV1, oxidative stress, inflammation

04.002 The inflammatory response is an important part of the myonecrosis induced by snake venoms: neutralization by wedelolactone and dexamethasone.

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In the present work, we investigated de acute damage and inflammatory response induced by different worldwide distributed snakes venoms. We observed that snake venoms from *Bothrops, Crotalus, Agkistrodon, Naja, Lachesis* and *Echis* genus induced muscular damage, demonstrated by the fall in this enzyme content in the *Extensor digitorum longus* (EDL) of mice, associated with a inflammatory response, demonstrated by the presence of mieloperoxidase (MPO) enzyme in these muscles. A systemic response to *B. jararacussu* (1.0 mg/Kg) consisted of leukocytosis with important rise in blood neutrophils, T and B lymphocytes, and also the rise in the plasmatic levels of neutrophil-derived microvesicles. We also investigated the antiophidic effect of the *Eclipta prostrata* (EP) crude extract and of wedelolactone (WED), one of EP main components, on the inflammatory response induced by snake venom. The EP extract (50.0 mg/Kg) was efficient against the damage associated with the inflammatory response induced by 1.0 mg/Kg of *B. jararacussu, C. viridis viridis* and *N. naja* snake venoms, reducing in 79.0 %, 51.8 % and 51.6 %, respectively, the fall in EDL muscle CK content, and reducing in 42.5 %, 76.7 % e 42.5 %, respectively, the rise in mice EDL muscle MPO. EP extract did not reduced the leukocytosis induced by *B. jararacussu* venom (1.0 mg/Kg), but altered the proportion in the leukocytes types, reducing cytotoxic lymphocytes (T CD8+), maintaining auxiliary lymphocytes (T CD4+) and rising mature B lymphocytes blood levels in mice. WED (1.0 mg/Kg) reduced *B. jararacussu* and *N. naja* snake venoms miotoxicity. *In vitro*, EP extract and WED efficiently reduced the phospholipase activity present in 14 different snake venoms studied. Best results with EP extract (300.0 µg/mL) was against *E. carinatus* venom (10.0.µg/ml), that keep only 5.7 % of its original phospholipase activity. Meanwhile, *B. jararaca* (1.0 mg/Kg) in the presence of WED (100,0 µM) keep only 16,7 % of its original activity. The EP extract also reduced the CK rate release rise from the isolated EDL muscle bathed 25.0 µg/mL of *B. jararacussu, B. atrox, C. viridis viridis, E. carinatus* e *N. naja* snake venoms. All together our results show that inflammation is at least in part responsible for the local tissue damage induced by different worldwide distributed snakes, and also that EP extract and WED are potential candidates in treatment of snake bites.

04.003 Effect of n-acetylcysteine in hepatic oxidative stress in mice with severe ulcerative colitis. Andrade KQ[1], Araújo ORP[2], Martins ASP[3], Moura FA[4], Azevedo MLSG[5], Goulart MOF[2] [1]IBCCF-UFRJ, [2]IQB-UFAL, [3] ICBS-UFAL, [4]FANUT-UFAL – Nutrição, [5]UFAL – Química e Biotecnologia

Introduction: Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) that affects the large intestine. It can trigger liver damage. Inflammation and oxidative stress (OS) are the main mediators of this damage. N-acetylcysteine (NAC) has an antioxidant action and anti-inflammatory. Therefore, the present study aimed to evaluate the effect of NAC administration in mice with severe UC induced by dextran sulfate sodium (DSS). **Methods:** For this, Swiss male mice (n=18), 8 weeks old, were randomly conditioned according to the treatment group: Control (C) (n=6); Colitis (UC) (n=6) (DSS) (5% (m/v), orally, in the last 7 days of the total of 37 days of the experimental period); Colitis treated with NAC (NACc) (n=6) (150 mg/kg/day, orally, drinking water for 37 days, and induced colitis in the last 7 days). Approved by the Ethics Committee for Animal Use (CEUA) at Federal University of Alagoas (UFAL), under the number of process 45/2016. Dietary intake and weight was carried out weekly. The glycemic levels were measured. After the euthanasia, the absolute (WA) and relative (WR) weights of the liver were verified and, analyzes were performed on hepatic tissue: histological, oxidative stress and inflammation. ANOVA and Tukey test were used for parametric variables, and Kruskal-Wallis test and Dunn test for non-parametric variables. Up to 5% significance was adopted. **Results:** Loss of hepatic architecture was observed in the UC group, however, NAC didn't improve the damage. There wasn't change in body mass of the animals due to colitis and treatment with NAC ($p>0.05$), even with increase of dietary intake of the UC group in relation to group C, during the colitis induction phase ($p<0.05$). Treatment with NAC and colitis didn't alter WA and WR of the liver ($p>0.05$)**.** Regarding the biochemical data, was observed that colitis increased blood glucose ($p<0.001$, UC in relation to C), and the treatment with NAC didn't have a hypoglycemic effect ($p<0.001$, NACc in relation to C). MDA levels (malondialdehyde) of the UC group differed of the C group ($p<0.001$), showing an increase, however, the NAC didn't attenuate the lipid peroxidation ($p>0.05$ in relation to the UC). When NAC was given to mice of the NACc group, there was an increase in GSH levels (decreased in the UC group), similar to that presented by group C ($p<0.05$, UC vs NACc, C vs UC). In addition, NAC elevated the GSH/GSSG ratio (reduced and oxidized glutathione ratio) of the treated group compared to UC group ($p<0.05$). On the other hand, there was a significant decrease in SOD (superoxide dismutase) activity in the UC and NACc groups in relation to the control ($p<0.05$), possibly due to the statistically decreased hepatic MPO (myeloperoxidase) activity between the UC and NACc groups ($P<0.05$, C vs UC and NACc). Low IL-10 levels were found in the UC and NACc group ($p<0.05$, CUI and NACc vs C). On TNF-α levels, there was no significant difference between the groups ($p>0.05$). **Conclusion:** We conclude that NAC wasn't effective in attenuating liver damage. **Financial support:** CAPES, CNPq, FAPEAL

04.004 Quercetin suppression of pulmonary fibrosis triggered by silica particles in Swiss-Webster mice. Guimarães FV, Ferreira TPT, Arantes ACS, Alexandre TL, Martins MA, Silva PMR Fiocruz – Inflamação

Introduction: Silicosis is a chronic lung disease caused by inhalation of silica particles, which is characterized by fibrosis and granuloma formation. There is no treatment for silicosis. Quercetin is a flavonoid widely found in nature that is known by its antioxidant and anti-inflammatory activities. In this study we evaluated the therapeutic activity of quercetin in the experimental model of silicosis in mice. **Methods**: Swiss-Webster mice were anesthetized and instilled with silica (10 mg) by intranasal via. Treatment with quercetin (2.5 - 10 mg/kg, po.) was performed daily, between day 21 and 27, and the analysis performed 1 day after the last dose. In some set of experiments reference antioxidant compound N-acetylcysteine (NAC) was used, following a similar experimental protocol. The parameters evaluated included i) pulmonary function (resistance and elastance) and airway hyper-reactivity to methacholine were analyzed by whole body invasive plethysmography; Ii) morphological changes in the lung tissue by classical histological techniques; Iii) quantification of inflammatory mediators cytokines/chemokines by ELISA and markers of oxidative stress by biochemistry and immunohistochemistry. Some *in vitro* assays were performed to evaluate the effect of quercetin on macrophage and fibroblast reactivity. All experimental procedures were performed in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (LW57/14). **Results**: We showed that silicotic mice exhibited an increased lung resistance and elastance, as well as airways hyper-reactivity. In parallel, we noted the presence of an inflammatory infiltrate, fibrosis and an extensive collagen deposition and granuloma formation in the silicotic lungs. We also noted an increase in the levels of cytokines (IL-1β, TNF-α and IL-6), chemokines (KC, MIP-1a, MIP-2 and MCP-1), reactive oxygen species and alteration of activity of some oxidative stress enzymes. Oral therapeutic administration of silicotic mice with quercetin reduced lung inflammation and fibrosis as well as decreased cytokine and chemokine production. Quercetin also exhibited antioxidant properties as attested by reduction ROS, isoprostane and MDA levels as well as SOD and GPx and NOX4 enzymes. Treatment with the antioxidant NAC reversed changes associated with the oxidative stress and also with some enzymes involved in the oxidative stress. By means of *in vitro* assays we observed the direct inhibitory activity of quercetin on alveolar macrophages (AMJ2C11 line) and pulmonary fibroblasts, which are important cells of the silicosis disease. **Conclusion**: Altogether, our results show that treatment with quercetin markedly reduced the compromised lung function in silicotic mice, suggesting that quercetin may be considered as a promising therapeutic tool for future application in cases of fibrotic chronic diseases such as silicosis. **Financial Support**: FIOCRUZ, CNPq, FAPERJ, CAPES.

04.005 Proteinase-activated Receptor (PAR)-2 Blockade Impairs Ovalbumin-induced Airway Inflammation Matos NA[1], Lima OCO[1], Rocha LK[1], Mattos MS[1], Alvarez ARP[2], Ferreira RG[2], Silva JF[1], Lemos VS[1], Alves-Filho JC[2], Russo RC[1], Tavares JC[1], Klein A[1] [1]ICB-UFMG – Farmacologia e Fisiologia, [2]FMRP-USP – Farmacologia

Introduction: Allergic asthma is an inflammatory disease of the airways, which is characterized by airflow's limitation due bronchoconstriction, bronchial hyperresponsiveness, altered vascular permeability, edema and eosinophil recruitment. The severity of the disease is directly related to the intensity of these symptoms, making it necessary to understand the mechanisms involved and to find ways of control these diseases. PAR-2 (G-protein-coupled receptor) has been implicated in mediating allergic airway inflammation once is expressed by many cells in the airways as epithelial, endothelial and macrophages. We have demonstrated that PAR-2 plays a role in eosinophil recruitment in experimental allergen-induced pleurisy supporting additional evidences for a role for PAR-2 in allergic diseases (Matos et al.,Eur J Pharmacol, 740, 627, 2014; Matos et al., Inflammation, 36, 1260, 2013) . However, the role of PAR-2 in airway inflammation has not been fully understood. In this work, we evaluated the effects of PAR-2 blockade in allergen-induced airway inflammation. **Methods:** Briefly, ovalbumin-sensitized BALB/c mice were challenged throughout the intranasal instillation of the immunogen (10µg) or PBS 9-11, 13, 15 and 17 days after and pretreated or not systemically with selective PAR-2 antagonist ENMD1068 (0.1–1.0mg/kg). Assessment of respiratory mechanics (by Forced Pulmonary Maneuver System®, Buxco Research Systems©), as well as bronchoalveolar lavage fluid (BALF) and lungs were obtained 30min-72h after challenging, depending on each protocol. BALF were assessed for leukocyte migration, *cytokines* levels (by *ELISA method) and* analysis of leukocyte PAR-2 expression (by Western blot). M2 lung macrophages were identified by flow cytometry. V*ascular permeability* assay, MPO and EPO activity by spectrophotometric absorbance measurements. **Results:** ENMD1068 significantly reduced the number of airway inflammatory cells, including eosinophils, in BALF following ovalbumin (OVA) challenge when compared to OVA-treated mice in different times analyzed ($p<0.05$). ENMD1068 decreased PAR-2 expression ($p<0.05$) and levels of KC, IL-6, CCL5 in BALF ($p<0.05$), as well extravasation of Evans Blue in lung tissue 2h after challenging. On the other hand, ENMD1068 increased significantly IL-10 levels 4h after immunogen when compared with OVA-treated mice ($p<0.05$). ENMD1068 also reduced the numbers of M2 lung macrophages ($p<0.001$), MPO and EPO activity ($p<0.01$) in pulmonary parenchyma, and decreased loss of dynamic compliance and reduced the lung resistance in response to methacholine ($p<00.5$) when compared to OVA-treated mice 48h after challenging. **Conclusion:** Our results demonstrated that a selective blockage of PAR-2 receptors by ENMD1068 could prevent development of allergen-induced airway inflammation and suggest a modulatory role of the activation of PAR-2 on cytokines production, vascular permeability, macrophages differentiation and pulmonary remodeling. ENMD1068 may be used as a therapeutic target to the suppression of allergic inflammation diseases. **Financial Support:** CAPES, CNPq and FAPEMIG/Brazil. (UFMG Ethics´s Committee for Animal Use: certificated number 348/2014).

04.006 Immunometabolic reprograming play a critical role in the pathogenesis of psoriasis through a PKM2-dependent mechanism. Veras FP[1], Melo B[1], Prado D[1], Norbiato TS[1], Melo P[1], Costa L[2], Cecilio N[1], Publio G[1], Schüller R[4], Nikolaev A[3], Lima D[4], Alves M[5], Cunha TM[1], Nakaya H[1,4], Sales KU[5], Souza C[2], Cunha FQ[1], Waisman A[3], Alves-Filho JC[1] [1]CRID-FMRP-USP – Pharmacology, [2]FMRP-USP – Dermatology, [3]University Medical Center of the Johannes Gutenberg – Molecular Medicine, [4]FCF-USP – Clinical and Toxicological Analyses, [5]FMRP-USP – Cell, Molecular Biology and Biopathogenic Agents

Introduction: Psoriasis (Ps) is a skin inflammatory disease, characterized by hyperproliferation of keratinocytes (KC) and infiltration of inflammatory cells. Metabolic pathways in the immune system have brought the immunometabolism studies highlights. In this context, pyruvate kinase M2 (PKM2), a glycolysis enzyme, can also translocate into the nucleus and control inflammatory gene expression. Herein, we investigated the role of metabolic changes in the development of psoriasis. **Methods AND Results:** To investigate the role of metabolic changes in the development of Ps, we induced Ps-like by imiquimod (IMQ) in mice and treated them daily with 2-deoxy-glucose (2-DG), a glycolysis inhibitor (0.5 g.kg-1, i.p). 2-DG-treatment ameliorates clinical score. Next, to explore the role of PKM2 in Ps, we employed a model of Ps-like induced by topical application of IMQ and spontaneous transgenic models: K14-IL-$17^{ind/+}$ and DC-IL-$17^{ind/+}$. After development of Ps, we showed increased expression of PKM2, determined by immunostaining (IF), in all models of Ps. To determine the role of PKM2 in the development of Ps, we induced Ps-like by IMQ in mice and treated them daily with shikonin (SKN), a PKM2 inhibitor (4 mg.kg-1, i.p). SKN-treatment decreases cells infiltration and epidermal thickness. Next, we evaluates inflammatory gene expression (*Il17, Il22, Il23*, *S100a9, S100a8, Lcn2* and *K17*) by qPCR after PKM2 inhibition. Therefore, we assessed the levels of cytokines, TNF-α, IL-17, IL-22 and IL-23 by ELISA. Interestingly, treatment with SKN reduced strongly mediators associated with psoriasis in compared with vehicle treatment. To corroborate the phenotype above, we evaluated population of Th17 cells ($IL17^{+}CD4^{+}$) by FACS. We found that SKN-treatment decreases Th17 cells frequency after Ps induction. Next, to assess whether metabolic changes plays a role in human psoriasis we examined data of large-scale gene expression from human samples. We found overexpression of glycolytic genes, including PKM2, in psoriatic lesioned skin. Moreover, PKM2 is correlated with Th17-related cytokines and KC expression (*IL17A, IL22, IL23, K17, K14, LCN2* and *S100A9*). Indeed, IF and Western Blot analysis confirmed that PKM2 is overexpressed in lesioned skin. Finally, we evaluated the role of PKM2 on the human KC (HaCAT cells). We observed that PKM2 is overexpressed after IMQ, IL-17 or IL-22 stimulation. Indeed, PKM2 contributes for release of cytokines TNF-α and IL-8 in KC since that PKM2 inhibition led to a decrease theses mediators. All protocols were conducted in accordance with ethical guidelines. Animals (167/2015) / Human (CAAE 56869316.3.0000.5440). **Conclusion:** Accordingly, the data depicted above suggest that PKM2 is crucial for development and maintenance of psoriasis. In summary, our findings provide a new insight into the development of Ps. **Financial Support: FAPESP, CNPq, CAPES**

04.007 Loss of capacity to up-regulate MKP-1 and down-regulate PP38 and GATA-3 underline glucocorticoid insensitivity in allergic asthma changes in A/J mice. Cotias AC[1], Pão CRR[1], Daleprane JB[2], Couto GC[1], Anjos-Valotta EA[1], Cordeiro RSB[1], Silva PMR[1], Serra MF[1], Martins MA[1] [1]Fiocruz – Fisiologia e Farmacodinâmica, [2]UERJ – Nutrição Básica e Experimental

Introduction: The importance of developing new animal models to assess the pathogenesis of glucocorticoid-insensitive asthma has been stressed. Because of the asthma-prone background of A/J mice, we hypothesized that asthma changes in mice of this strain would be or become resistant to glucocorticoids under repeated exposures to the allergen. **Methods:** A/J mice were challenged with ovalbumin for two or four consecutive days, starting on day 19 post- sensitization. Oral dexamethasone or inhaled budesonide were given 1 h before challenge, and analyses were done 24 h after the last challenge. Lung function, leukocyte infiltration, tissue remodelling, transcription factors, and cytokines were the major readouts. Western blotting was used to investigate MKP-1, pp38 and GATA-3 expression. (CEUA license # L-030/15). **Results:** A/J mice subjected to two daily challenges reacted with airway hyper-reactivity, sub-epithelial fibrosis and marked accumulation of eosinophils in both BAL fluid and peribronchial space, all of which being clearly sensitive to dexamethasone and budesonide. Conversely, under four provocations, most of these changes were steroid-resistant in A/J mice but not in BALB/c. Also, elevations in lung tissue levels of pro-inflammatory mediators, including IL-4, IL-13 and IL-17, were responsive to steroids following two, but not four ovalbumin challenges in A/J mice. Finally, the efficacy of glucocorticoid treatment to up-regulate MKP-1 expression (0,62±0,03 to 1,76±0,03- Mean ± SEM, n=4) and down-regulate pp38 (0,86±0,12 to 0,6±0,06-Mean ± SEM, n=4) and GATA-3 expression (7300±586,5 to 4553±588,9- Mean ± SEM, n=4) was seen after two but not four provocations in this model. **Conclusion:** These findings suggest that steroid efficacy may decrease in A/J mice subjected to repetitive allergen provocations. The glucocorticoid insensitivity is strain-specific and associated with the loss of steroid capacity to modulate pivotal regulatory protein such as GATA-3, pp38 and MKP-1 in the lung. **Financial Support:** CNPq, FAPERJ and CAPES.

04.008 Resolvin E1 reduces rat paw edema and nociception locally through leukotriene B_4 receptor type one. Fonseca FCS, Turchetti-Maia RMM, Francischi JN
UFMG – Farmacologia

Introduction: The discovery of specialized pro-resolving lipid mediators, also known as lipoxins, resolvins, protectins and maresins (Serhan et al., 2015), challenged our comprehension of the inflammatory process. The aim of the present work was to verify the mechanisms involved in the biological activity presented by resolvin E1 (RvE1) using the rat paw edema and mechanical nociceptive models. **Methods:** Paw edema (Δ increase in volume, in ml) and mechanical nociception (Δ threshold, in g) were measured at 0, ¼, ½ , 1, 2, 3, 4, 6 and 24h with an Ugo Basile plethysmometer and the Randall-Selitto analgesimeter, respectively, using male Holtzman rats (150-180g). RvE1 diluted in 5% ethanol was injected intraplantarly by subcutaneous route, 10 min before the phlogogenic stimuli, given at zero time. The used phlogogenic stimuli were: carrageenan (CG), prostaglandin E_2 (PGE_2), histamine (H), 5-hydroxytriptamine (5-HT) and substance P (SP) in doses known to induce paw edema and/or nociception. The selective antagonists of the leukotriene B_4 receptor type 1 (BLT1), and of the free fatty acid receptors 1 (FFAR1/GPR40) and 4 (FFAR4/GPR120), respectively, U-75302, GW1100 and AH7614, diluted with an ethanol-saline solution (vehicle) were given intraplantarly 5 min (in the case of U-75302) and 15 min (for the others) before the injection of RvE1. All injections, including controls, were made in a volume of 100 µL. The results were shown as the mean ± SEM of the area under the curve (AUC) for the time course for each group of animals (n=5/group). The statistical analysis used Student's t-test or one-way ANOVA, and difference between means was considered significant when $p<0.05$. **Results:** RvE1 reduced edema induced by CG (Veh+CG=4.9; RvE1+CG=2.9) but did not affect SP- and PGE_2-induced edema. Furthermore, RvE1 increased H and 5-HT edema. RvE1 also reduced CG- (Veh+CG=-488.6; RvE1+CG=-180.2), PGE_2-, 5-HT- and SP-induced nociceptive responses, without causing per se any response. The selective antagonist of the receptor BLT1 (U-75302), but not the antagonists of FFAR1 and FFAR4 (GW1100 and AH7614, respectively), reduced the anti-edematogenic (Veh+RvE1+CG=1.4; U-75302+RvE1+CG=2.2) and anti-nociceptive (Veh+RvE1+CG=-81.5; U-75302+RvE1+CG=-156.0) activity of RvE1 in the CG models studied. **Conclusion:** RvE1 was an effective anti-edematogenic and anti-nociceptive agent given peripherally and BLT1 receptors seem to be the signalling pathway involved in these actions. However, adverse effects, such as edema formation, can account for the potential side effects of RvE1 in the clinic. References: Serhan CN, Dalli J, Colas RA, Winkler JW, Chiang N. Protectins and maresins : New pro-resolving families of mediators in acute inflammation and resolution bioactive metabolome. Biochim Biophys Acta. 2015;1851:397-413. **Financial Support**: CNPq, CAPES and FAPEMIG Experimental procedures were approved by UFMG Ethics´s Committee for Animal Use (CEUA/UFMG, n#199/2014).

04.009 Analysis of regulatory role of 5- and 12-Lipoxygenase pathways in skeletal muscle regeneration events induced by a myotoxin. Damico MV[1], Zuntini ACS[1], Fortes-Dias CL[2], Spadacci-Morena DD[3], Moreira V[1] [1]Unifesp – Farmacologia, [2]Fundação Ezequiel Dias – Pesquisa e Desenvolvimento, [3]IBu – Fisiopatologia

Introduction: Skeletal muscle tissue is provided by coordinated regeneration capacity after injury. Both degenerative process followed by regenerative phase are often accompanied by inflammatory response characterized by influx of leukocytes and release of inflammatory mediators on local of injury. Evidence suggests that the nature, extension and prevalence of inflammatory mediators and cells have regulatory influence on the quality of tissue repair. In this context, the regulatory role of eicosanoids on injury and tissue repair, particularly those produced by 5- and 12-lipoxygenase (LOX) pathways, is still little known, justifying studies using in vivo experimental models. The aim of this study was to analyze the regulatory effects of eicosanoids produced by 5- and 12-LOX pathways on histological aspects of skeletal muscle degeneration and regeneration induced by a myotoxin. **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. After 30 min, distinct groups of mice received oral administration (p.o.) of MK-886 (MK), 5-LOX inhibitor (3mg/kg/100µL), or Baicalein (BA), 12-LOX inhibitor (20mg/kg/100µL), or vehicle (CMC 1%). After 6 and 24h or 3, 7, 14 and 21d, mice were sacrificed by cervical displacement and muscle was 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 - Retiga 2000R Camera). Percentage of injured fibers (%IF) and central nuclei (%CN), both calculated from total muscle fibers, and counting of cell influx (CI) into local of injury, were used as histological parameters. **Results:** In degenerative phase (6h to 3d), mice treated with CB/BA showed significant increase ($p<0,01$) of %FL (34,0±1,7%: 6h and 42,5±1,5%: 24h) when compared to group CB/CMC (22,0±0,2%: 6h and 25,7±0,7%: 24h). After 24h, CB/BA-treated group showed significant increase ($p<0,01$) of CI (3160,0±130,0 cells) in comparison with mice CB/CMC (2258,7±454,3 cells). After 3d, the treatment with CB/MK significantly reduced %FL (5,0±0%) and CI (865,0±98,0 cells) when compared to CB/CMC-treated mice (18,5±1,5% and 2366,0±193 cells). In regenerative phase (7 to 21d), mice treated with CB/BA did not show significant difference of %NC ($p<0,01$) when compared to mice treated with CB/CMC, whereas mice treated with CB/MK showed significant increase ($p<0,01$) of %NC (57,0±0,8: 7d) in comparison with group treated with CB/CMC (30,7±0,7: 7d). **Conclusion:** For the first time, it was show that 5- and 12-LOX pathways promote antagonistic role on the regulation of muscular degeneration. Data suggest that 12-LOX-derived mediators down-regulate the elements of muscular degeneration, whereas 5-LOX-derived eicosanoids may exert up-regulation of mechanisms that lead to muscular injury. Regarding the presence of central nuclei on muscle fiber, results suggest that 5-LOX-derived mediators down-regulate the progress of tissue regeneration. **Financial Support**: FAPESP and CNPq. Ethics Committee in Animal Experimentation: 4892220616.

04.010 Anti-inflammatory effect of trans-resveratrol loaded lipid-core nanocapsules on acute lung injury induced by LPS in mice. Oliveira MTP[1], Souza ET, Coutinho DS[1], Guterres SS[2], Pohlmann AR[2], Silva Martins PMR[1], Martins MA[1], Bernardi A[1] [1]Fiocruz, [2]UFRGS

Introduction: Resveratrol (RSV) has a broad spectrum of biological activities, including anti-inflammatory and anti-oxidant effects. However, therapeutic application of RSV's beneficial effects remains very limited due to its low solubility, reduced stability, intense metabolism and high rate of excretion. In order to overcome these limitations, RSV was incorporated into polymeric lipid core nanocapsules (RSV-LNC). Nanometric formulations improve pharmacokinetic limitations, allowing sustained release and reaching target tissues. We tested RSV-LNC in an experimental model of acute respiratory distress syndrome (ARDS), considered to be a serious and potentially fatal medical condition, currently without effective therapy. **Methods:** A/J mice were orally treated with RSV-LNC or free molecule (RSV) (2.5, 5 or 10 mg/kg), 4 h before or 6 h after LPS nasal instillation (25 µg/mouse) in pre- and post-treatment conditions, respectively. Nanocapsules without resveratrol (LNC) were used as a control. Twenty-four hour after LPS provocation, pulmonary elastance (accessed by invasive plethysmography), leukocyte infiltrate (bronchoalveolar lavage), lung tissue levels of myeloperoxidase (MPO) (ortho-dianisidine method), cytokines (ELISA assay), lung histological changes (Hematoxylin-Eosin staining) as well as lung concentration of RSV (HPLC) were evaluated. **Results:** Data showed a significant inflammation improvement in the animals treated with RSV-LNC when compared to the LPS group in both pre- and post-treatment conditions. We observed a robust decreased in leukocyte migration, mainly neutrophils, MPO activity reduction, minor tissue levels of cytokines (IL-6, KC, RANTES and MIP-1) and reduced tissue damage, resulting in lung function improvement. Treatment with RSV or LNC did not show anti-inflammatory activity. HPLC analysis revealed that only the RSV-LNC treated groups showed significant amounts of RSV in lung tissue. **Conclusion:** The data allow us to conclude that the incorporation of RSV into polymeric nanocapsules significantly increased bioavailability, improving its biological activity in the modulation of ARDS model. **References:** Frémont FL. Minireview: biological effects of resveratrol. Life Sci. 2000, 66, 663–673; Frozza RL, et al. Incorporation of resveratrol into lipid-core nanocapsules improves its cerebral bioavailability and reduces the Aβ-induced toxicity. Alzheimers Dement. 2011;7(4):S114; Jager E, et al. Sustained release from lipid-core nanocapsules by varying the core viscosity and the particle surface area. J Biomed. Nanotechnol. 2009, Feb;5(1):130-40; Fanelli V, et al. Acute respiratory distress syndrome: new definition, current and future therapeutic options. J Thorac Dis. 2013 Jun;5(3):326-34; **Financial support:** CNPq, CAPES and FAPERJ. **Animal research ethical committee process number:** L-006/2016.

04.011 Hydrogen Sulfide (H2S) cutaneous biosynthesis is impaired in psoriasis: Role of exogenous supply of H2S. Rodrigues L[1], Schimidt T[1], Cerqueira ARA[1], Soares AG[1], Whiteman M[2], Teixeira SA[1], Muscará MN[1], Costa SK[1] [1]ICB-USP – Farmacologia, [2]University of Exeter – St. Luke's Campus

Introduction: Hydrogen sulfide (H_2S) has emerged as a novel biological mediator associated with inflammatory and sensitive (eg. pain, itch) responses (Wang, Physiol Rev.92(2):791, 2012). We have recently shown that treatment with H_2S donors ameliorates pruritus and acute inflammation in mice dorsal skin via histaminergic and non-histaminergic-dependent pathways (Rodrigues, Pharmacol Res.115:255, 2017; Coavoy-Sánchez, Pharmacol Res.113:686, 2017). In despite of the protective effects of H_2S on cutaneous acute inflammation, its effect on chronic pruritus and inflammatory processes such as psoriasis is poorly known. In this study we aimed to investigate the effects of the slow-releasing H_2S donor (GYY4137) in a murine experimental model of psoriasis. **Methods**: Experiments were carried out on male BALB/c mice (20-25g). The psoriasis-like disease was induced in the mouse shaved dorsal skin and ear surfaces by daily application of imiquimod 5% (IMQ, 65 mg/mouse; Germed Pharmaceuticals, Brazil; n=5) or the equivalent amount of vehicle (vaseline, 65 mg/animal) for 5 days (van der Fits, J Immunol.182(9):5836, 2009). Control and psoriatic-like mice were daily treated, via i.p., with the slow releasing H_2S donor GYY4137 (25, 50 or 100 mg/kg) or its vehicle (saline). The severity of Psoriasis Area Severity Index (PASI) was simultaneously scored from 0 to 4 over six days, whereas ear thickness (oedema) was assessed via digital caliper and spontaneous itching was recorded for 30 min on day 6 (Sony Handycam DCR-PJ6, Japan). Following euthanasia, biological samples were collected and leucocyte counts, cytokines concentration and enzymatic activity were analysed. Statistical analysis was performed via ANOVA plus Dunnett's test.

Results: The treatment with IMQ induced signs and symptoms (itch) that closely resemble human plaque type psoriasis, including erythema, scaling, increased skin thickening and spontaneous itching behavior on the last day. This effect was paralleled by systemic and splenic increase of leukocytes counts and pro-inflammatory cytokines (TNF-□, IL-1□, IL-6 e INF-□) but reduced concentration of IL-10. Mice with psoriasis exhibited reduced H_2S cutaneous production and enzymatic expression of cystathionine-□-synthase (CBS), an enzyme linked to H_2S synthesis. The exogenous supply of H_2S by treating the animals with GYY4137 led to a significant inhibition of the PASI index, itching behavior, total leukocyte counts in blood and spleen, augmented concentration of IL-10 and antioxidant enzymes activity (eg. glutathione peroxidase, reductase, S-transferase). **Conclusion**: We show for the first time that reduced biosynthesis of cutaneous H_2S and concomitantly downregulation of CBS expression is linked to psoriasis, and the local delivery of exogenous H_2S shows the potential for reducing psoriasis-related PASI index, local / systemic increased leukocyte infiltration, itching behavior and proves to be safe in this animal model. The involved mechanisms seem to be correlated with augmented production of IL-10 and antioxidants activity. **Acknowledgments**: CNPq, CAPES and FAPESP for **Financial Support**. Ethic committee process number: 100/2013/CEUA

04.012 Fructose 1,6-bisphosphate, a glycolytic metabolite, tunes the metabolic reprogram of pro-inflammatory macrophages. Viacava PR[1], Nascimento DRB, Luiz JPM, Veras FP, Ferreira RG, Vitorino CA, Peres RS, Cunha FQ, Cunha TM, Alves-Filho JCF FMRP-USP

Fructose 1.6-bisphosphate (FBP) is an endogenous intermediate of glycolytic pathway. Exogenous administration of FBP showed to exert protective effects in injury models, which is attributed to sustain glycolysis and increase ATP production. Moreover, in arthritis model, treatment with FBP has also been shown to exert anti-inflammatory effects via production of adenosine, although its mechanism is not fully understood. We hypothesized that metabolic reprogramming by FBP could modulate the macrophage inflammatory response. Here, we show that FBP administration enhances IL-10 production while reduces the production of IL-1β and IL-6 by LPS-activated macrophages. The presence of FBP in macrophage increases the cellular metabolism resulting in higher production of lactate, ATP and IL-10 dependent manner the glycolytic pathway. Upon activation, we observe increased levels of the metabolics byproducts such as glycolysis or oxidative phosphorylation, that are reduced in the presence a competitor of hexokinase (2-DG) or GAPDH inhibitor (IAA). However, in the presence of FBP the production of metabolites is increased and remains higher even when occurs inhibition the glycolytic pathway with 2-DG, but when we use the IAA, which is an inhibitor at downstream FBP, we observed a reduction in the production of metabolites. Mechanistically, FBP boosts glycolysis pathway increasing synthesis and secretion of ATP, which act as an autocrine signal that self-limit the macrophage activation state due its rapid catabolism into adenosine. We also observed FBP was able to induce the production of adenosine and that this production was dampened when glycolytic pathway was blocked with IAA. Moreover, the inhibition of Pannexin-1, an ATP-releasing channel, or ectonucleotidases CD39 and CD73 blocked the enhanced production of IL-10 by FBP. We also found that inhibition of adenosine receptor A2a (A2aR) or deficient macrophages (A2aR KO) blocked the enhanced production of IL-10 by FBP. In line, FBP failed to enhance IL-10 production in LPS-activated macrophages from A2aR or Pannexin-1 KO mice and also in the presence of adenosine deaminase. Finally, in colitis and peritonitis model, treatment with FBP was able to reduce the score of the disease, although the levels of IL-10 in colon, serum and peritoneal lavage were increased. Taken together, these data implicate that FBP as a key molecule in the cellular metabolism. When stimulated, macrophages depend on this molecule to enhance the production and release of ATP that will be further dephosphorylated into adenosine by CD39/CD73 pathway, self-limiting their activation state, increasing the IL-10 production and possibly regulating the inflammatory process by enhances adenosine levels.

04.013 Effects of Resolvin D1 treatment on eosinophilic inflammation in lean and obese mice. Tavares EBG, André DM, Calixto MC, Antunes E Unicamp – Farmacologia

Introduction: Asthma is a public health problem with high global prevalence. It is a chronic disease of the airways, characterized by accumulation of inflammatory cells, especially eosinophils. Obesity is considered a disease of alarming proportions, being an aggravating factor for pre-existing asthma (Mosen et al., 2008). There is still no consensus on the causality and mechanisms involved in this relationship. During inflammation, docosahexaenoic acid (DHA), an omega-3 derivative, undergoes enzymatic transformations leading to generation of resolution mediators, including D-series resolvins (Serhan et al., 2013). A previous study in asthmatic mice showed that resolvin D1 (RvD1) inhibits neutrophils and production of cytokines, thus exhibiting anti-inflammatory and pro-resolution actions in the airways (Rogerio et al., 2012). It is known that the inflammatory profile in obese asthmatic mice greatly differ from the lean group, suggesting that the resolution process in obese animal is impaired. In the present study we propose to study the effect of treatment with RvD1 on the exacerbation of asthma in high-fat diet-fed obese mice. Methodology: Male C57BL/6 mice fed with standard diet or high fat diet for 12 weeks (Calixto et al., 2010). At the 10th and 11th weeks, the animals were sensitized with ovalbumin (OVA). Next, the animals were challenged for 4 days with OVA at week 12. Groups of obese mice and controls were treated with RvD1 (5 µg/kg intraperitoneally, concomitant with the 4-days OVA challenge). At 4 and 8 days after OVA challenge, bronchoalveolar lavage (BAL) fluid was performed and lungs were collected for histological analysis. **Results:** In lean animals at 4 days, RvD1 significantly decreased the infiltration of total inflammatory cells and eosinophils in the perivascular and peribronchiolar compartments of the lung tissue, and concomitantly increased the eosinophilic infiltrate in BAL. At 8 days, a significant decrease of the inflammatory infiltrate was observed in the three compartments studied. The levels of IL-5 and eotaxin as well as the apoptotic cell number decreased whereas IL-10 levels increased significantly by RvD1 treatment. In obese mice RvD1 treatment did not modify the total cellular infiltrate in BAL at 4 and 8 days. With regard the eosinophilic infiltrate at 4 days, there was a reduction by RvD1 in the perivascular compartment, increasing significantly at 8 days. In the peribronchiolar compartment, the eosinophilic infiltrate significantly increased by RvD1 at 4 and 8 days. An increase of eosinophil number by RvD1 was also observed at 8 days. The levels of IL-5, IL4 and IL-10 in BAL as well as the apoptotic cell number were not altered by RvD1 treatment. **Conclusion:** We confirm here that RvD1 display an anti-inflammatory and pro-resolution action in airways of allergic lean mice. However, in obese mice, RvD1 treatment generally enhanced the airways cell infiltrate. New hypothesis may be raised to explain the effects of RvD1 in asthma of obese individuals. **Financial Support**: CNPq The experimental protocols have been approved by the Ethics Committee of UNICAMP (N$^{o.}$ 3493-1). References: 1. Mosen DM, et al. JACI 122: 507, 2008. 2. Serhan CN, et al. Curr. Opin. Pharmacol 13: 632, 2013. 3. Rogerio AP, et al. The JI 189: 1983, 2012. 4. Calixto MC, et al. BJP 159: 617, 2010.

04.014 ERK5 is a molecular switch that controls the fate between Th17 and Treg cell differentiation and development of experimental autoimmune encephalomyelitis. Prado DS[1], Damasceno LEA[1], Ferreira RG[1], Cunha TM[1], Cunha FQ[1], Ryffel B[2], Alves-Filho JC[1] [1]FMRP-USP – Farmacologia, [2]CNRS – Immunologie et Neurogénétique Expérimentales et Moléculaires

Introduction: ERK5 is an atypical member of MAPK family that also exerts noncanonical functions such as act as a scaffold protein or co-transcription factor. It has been shown that ERK5 is important for TGF-β signalling in fibrosblast and epithelial cells. In line, it is very well established that TGF-β is critical for Treg and Th17 differentiation, being important to control autoimmunity development. We hypothesized that ERK5 could modulate Treg and Th17 differentiation playing a key role in the fate of autoimmunity **Aim:** to evaluate the role of ERK5 on $CD4^+$ T cell differentiation and experimental autoimmune encephalomyelitis (EAE) development. **Methods:** $CD4^+CD25^-$ T cells purified from C57BL/6, $ERK5^{flox/flox}$ or $CD4^{cre}ERK5^{flox/flox}$ mice were cultured under Treg- or Th17-polarizing conditions, then their differentiation was analysed by flow cytometry. In order to check the role of ERK5 in Treg and Th17 differentiation, ERK5 pathway was blocked with different inhibitors, such as BIX 02189 (0,3, 1 or 3 µM; MEK5 inhibitor), XMD 8-92 or ERK5-IN-1 (0,3, 1 or 3 µM; 0,1, 0,3 or 1 µM, respectively; ERK5 inhibitors). The role of ERK5 in the pathogenesis of autoimmune disease was investigated by inducing EAE in mice. **Results:** we found that pharmacological inhibition or genetic deficiency of ERK5 in CD4 T cells decreased Treg cell differentiation, whereas Th17 polarization was augmented. Moreover, $CD4^{cre}ERK5^{flox/flox}$ mice developed more severe EAE than control $ERK5^{flox/flox}$ mice, characterized by an increase of clinical score and Th17 cell frequency, as well as a reduction of Treg cells in draining lymph nodes and spleen. **Conclusion:** our study reveals a novel role of ERK5 in modulating Treg/Th17 differentiation and attenuating the severity of EAE. Therefore, modulation of ERK5 pathway could be a potential therapeutic target for autoimmune diseases, including multiple sclerosis. **Financial support:** FAPESP, CRID, CNPq and CAPES. **Animal Research Ethical Committee from Ribeirão Preto Medical School:** 69/2017.

04.015 Involvement of vasoactive amines in the anti-inflammatory mechanisms of sulfated polysaccharides from the alga Gracilaria birdiae. Soares VVM[1], Frota AF[1], Castro LGZ[1], Souza RM[1], Coura CO, Benevides NMB[1] [1]UFC – Bioquímica e Biologia Molecular

Inflammation is an organism's protective reaction to several stimuli, such as microbial infection, chemical irritants and tissue injury. The early phase of acute inflammation involves the cellular influx associated with the release of mediators such as histamine, serotonin and bradykinin. Seaweeds are rich sources of diverse bioactive compounds, such as sulfated polysaccharides (SPs). These polymers are recognized as having a great number of biological activities, including anticoagulant, antiviral and anti-inflammatory. Anti-inflammatory effects of a sulfated polysaccharidic fraction obtained from red seaweed *Gracilaria birdiae* (Gb-FI) have been previously reported, however, their mechanisms of action are still unknown. Thus, this study aimed to evaluate the anti-inflammatory effect of Gb-FI and to investigate the possible involvement of vasoactive amines in this effect. Total sulfated polysaccharides were extracted by enzymatic digestion and fractioned by ion exchange chromatography on DEAE-cellulose column. The participation of vasoactive amines was investigated using the paw edema model induced by different inflammatory stimuli in rats. Male Wistar rats were pretreated with a subcutaneous injection (s.c) of sterile saline (0.9%), Gb-FI (5, 10 or 20 mg/kg) or dexamethasone (1 mg/kg, s.c.) 1h before receiving an injection of carrageenan (700 µg/paw s.c.), histamine (100µg/paw s.c.), serotonin (20 µg/paw s.c.) or bradykinin (30µg/paw s.c.) into the right paws. The paw volume was measured immediately before (zero time) the stimulus and at selected time intervals (0.5, 1, 2, 3 and 4 h) after the stimulus using a plethysmometer. It should be noted that all animal experimental procedures and protocols were approved by Ethics Committee of the Federal University of Ceará, Fortaleza, Brazil (CEPA n◦71/2012). Carrageenan (Cg) induced intense paw edema, which reached a maximum level at 3 h after injection. Gb-FI (5, 10 or 20 mg/kg, s.c.) significantly reduced edema formation, particularly in the third hour, by 75.9%, 65.3%, 61.5%, respectively. Dexamethasone also inhibited edema by 86.5%. As the dose of 5 mg/kg was more efficient in reducing Cg induced paw edema at all-time intervals was used in the subsequent assays, in which Gb-FI (5 mg/kg) reduced the edematogenic effect elicited by histamine at 0.5 h; 1 h and 2 h intervals ($p<0.001$, $p<0.001$ and $p<0.05$, respectively) and serotonin at 0.5 h and 1 h intervals ($p<0.05$ and $p<0.001$, respectively), but was ineffective on bradykinin-induced paw edema. A sulfated polysaccharidic fraction from the red seaweed *G. birdiae* exhibit potent anti-inflammatory activity via negative modulation of histamine and serotonin in the carrageenan-induced paw edema, showing involvement of this vasoactive amines in the mechanism of action of this sulfated polysaccharide. This work was supported by Coordination for the Improvement of Higher Education Personnel (CAPES).

04.016 Signaling pathway involved in the inhibitory effect of tumor necrosis factor alpha on platelet aggregation of rats: Role of SRC, PKC, IKK and MAP Kinases. Bonfitto PHL, Naime ACA, Bueno PI, Antunes E, Marcondes S FCM-Unicamp – Farmacologia

Introduction: Platelets are important cells for the maintenance of hemostasis and for thrombus formation. They are also involved in inflammatory processes such as sepsis and atherosclerosis. Tumor necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine that leads to multiple effects on different cells, through activation of TNFR1 and TNFR2 receptors. Studies investigating the modulation of platelet activity by TNF-α are rare and controversial, and the results show either stimulatory or inhibitory effects. Previously, we have shown that TNF-α dose- and time-dependent inhibits ADP-induced platelet aggregation, but the signaling pathways involved on the inhibitory effect of this cytokine were not studied yet. Therefore, in the present work we investigated the role of IKK, ROS, Src, PKC, JNK, p38MAPK and ERK 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. Aggregation assays were carried out incubating platelets for 30 min with TNF-α (100pg/ml). In some experiments, the platelets were pre-incubated for 3 min with the inhibitors of IKK, PKCδ, PKCε, c-Src, JNK, ERK, p38MAPK, or with the ROS scavengers PEG-SOD and PEG-catalase before TNF addition. Platelet aggregation was measured in a two channel aggregometer (Chronolog Lumi-Aggregometer). Platelet viability was determined using MTT. **Results:** TNF-α (100pg/ml, 30min) significantly reduced ADP (5μM) induced platelet aggregation (inhibition of 70±7%), without affecting platelet viability. The effect of TNF-α on aggregation was not affected by the inhibitor of c-Src (PP2, 10μM). The inhibitory effect of TNF-α was decreased 112% by the selective inhibition of PKCε (SC3095, 1μm) and PKCδ (rottlerin, 5μM). However, non-selective inhibition of PKC (GF109203X, 10μM) did not modify the effect of the cytokine. Inhibition of IKK (IKK16, 0,1 μM) increased the inhibitory effect of TNF-α (61 ± 9% and 91±7% of inhibition in the absence or in presence of IKK16, respectively). Platelet viability was not modified by IKK16. The inhibition of p38MAPK (SB203580, 1μM), JNK (SP600125, 10nM) or ERK (FR180204, 30μM) increased the inhibitory effect of the cytokin (inhibition of 48,04±15%, 68,06±8%, 84,79±6% and 93,59±2% for the TNF-α alone or in the presence of SB203580, SP600125 and FR180204 respectively). Likewise, PEG-SOD (30U/ml) or PEG-catalase (300U/ml) augmented the inhibition of aggregation by the cytokin (inhibition of 65±10%, 85±7% and 86±14% for TNF-α alone, or in the presence of PEG-SOD or PEG-catalase respectively). **Conclusions:** The inhibitory effect of TNF-α on platelet aggregation is independent of IKK, c-Src, ERK, p38MAPK, JNK activation or ROS formation. However, the signaling pathway involving PKCε and PKCδ takes part in the inhibitory effect of TNF-α on ADP-induced platelet aggregation. **Financial support:** FAPESP**,** CNPQ **Research approval process number:** 3709-1

04.017 Blends of chitosan/polivinilalchool/macauba pulp oil in the wound cicatrization. Duarte LC[1], Silva GGO[2], Albuquerque TB[1], Moreno SE[2], Domingues NLC[3] [1]UFGD – Biotecnologia, [2]UCDB – Biotecnologia, [3]UFGD – Química Orgânica

Acrocomia aculeata (Jacq.) Lodd. popularly known as macaúba, is an economically important species of palm trees typical of the area between Cerrado and the Amazon forest. This plant is widely used as a source of food; however, its therapeutic properties are poorly understood. The beneficial effects of some palm tree species have been shown to be effective for promoting human health since their composition is a rich source of unsaturated tocopherols, carotenoids, antioxidants, phenolic compounds and fatty acids. In order to propose a new application of macaúba pulp oil we have developed a blend aiming to wound cicatrization. In this sense aiming to improve a chemical, mechanical and biological properties we used chitosan which is a biopolymer with properties, functions and applications especially in biomedical area due to its biocompatibility, biodegradability and non-toxicity, apart from it antimicrobial activity and low immunogenicity. Macauba pulp oil (MPO) was obtained by extraction with hexane at 40-60ºC in a Soxhlet apparatus. After was formulated a chitosan/polivinilalchool (CP – concentration 75% / 25% w/w respectively) and MPO blends (CP - blank blend obtained without oil and CP/MPO - blend with 20% (w/w of oil) that were characterized by UV–Vis, Fourier transform infrared spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). After the characterization study, the blends were evaluated at the wound healing in Swiss mice, as follow described. Two surgical excisions were made in the skin of each animal, using a histological punch. The experimental groups consisted of animals with ulcers treated with CP/MPO blends and animals with that ulcers were treated with CP and a non-treated group. The ulcers were evaluated in first day and after 2, 7 and 14 days post-surgery. The wound healing was measured by the program Image J and after statistical analysis by the Statistica 7 program. In relation to wound contraction area there was 47% more with CP/MPO compared CP blend 7 days post-surgery. Our results demonstrated that there was a statistically significant increase on the wound healing process in mice ulcer treated with CP/MPO compared with CP group for 7 days of treatment ($p<0,05$). The experimental procedures were in accordance with the Ethical Principles in Animal Research and approved by the Committee for Ethics in Animal Experimentation at the University Catolic Dom Bosco (number: 005/11). The authors thank FUNDECT for the financial assistance.

04.018 Effect of acetylcholine on the contractility of the ileum of wild type and P2X7-/- mice. Coutinho-Silva R[1], Andrade KQ[1], Silva CLM[2] [1]UFRJ – Biofísica, [2]UFRJ – Ciências Biomédicas

Introduction: In intestinal smooth muscle, acetylcholine produce contraction by activating muscarinic receptors. The muscarinic contraction generally is mediated via M3 subtype and depends on Ca^{2+} entry via Ca^{2+} channels opened by membrane depolarization. The purinergic system influences intestinal motility in the mammals being mediated by purine nucleotides and nucleosides via purinoceptors. In particular, the ionotropic P2X7 receptors are found on different types of cells within the gut. The present work aimed to investigate if there is any change in the contraction in response to acetylcholine of the ileum strip in mice P2X $7^{-/-}$. **Methods:** Animals were anesthetized. Segments of terminal ileum (1 cm long) were removed from C57BL/6 (WT) and P2X7$^{-/-}$ mice (2-5 months old) of either sex. The longitudinal muscle strips were vertically mounted in a 5-ml organ bath filled with Krebs-Ringer solution (in mM; NaCl 118.3; KCl 4,7; $CaCl_2$ 2,5; $MgSO_2$ 1,2; KH_2PO_4 1,2; $NaHCO_3$ 25; glucose 11.1, pH 7,4) which was bubbled with carbogen (5% CO_2 and 95% O_2) at 37 ^oC. The tissues were equilibrated under a tension of 1 g (~ 10 mN) for 30 min until baseline tension was stable. At the end of the equilibration period, to test tissue viability the ileum was contracted by adding KCl (100 mM) for ~5 min. Then the tissue was washed twice with the warm aerated Krebs in an interval of 15 minutes. To evaluate if there was muscarinic receptor desensitization, carbachol (10 µM), a muscarinic agonist, was used. Again the tissue was washed twice until baseline was restored. Dose-response curves to acetylcholine (0.01 – 30 µM) were constructed. Response to high concentration of KCl also were repeated at the end of the experiment and used for normalizing responses to acetylcholine. The contractile responses to the muscarinic agonists acetylcholine and carbachol, and KCl were measured using an isometric force transducer and expressed as a percentage of KCl-induced contraction. Data were analyzed by nonlinear regression to estimate potency (the agonist concentration that produces 50% of the maximal effect, EC_{50}) and efficacy (E_{max}). **Results:** It was found that acetylcholine produced a dose-dependent increase in contraction in both C57BL6 and P2X7$^{-/-}$ mice ileum preparations. These results corroborate previous data that acetylcholine induces contractility of the intestinal strip. The E_{max} of acetylcholine was 108 and 130 in the C57BL/6 and in the P2X7$^{-/-}$ groups, respectively. Moreover, the log EC_{50} of acetylcholine was -6.037 and -6.013 for P2X7$^{-/-}$ and C57BL6 mice, respectively. **Conclusion:** This preliminary study suggests that the absence of P2X7 receptor led to a tendency to have higher ileum contraction in response to acetylcholine as compared to the wild-type mice. **Key-words:** isolated ileum, intestinal motility, purinergic signaling. **Financial support and acknowledgments:** FAPERJ and CNPq **Number of process from Ethics Committee for Animal Use (CEUA) of UFRJ**: n° A12/17-076-15 **References:** BORNSTEIN, J. C. Purinergic mechanisms in the control of gastrointestinal motility. Purinergic Signalling, v. 4, p. 197–212, 2008. GUNS, P. J. D. F. Pharmacological characterization of nucleotide P2Y receptors on endothelial cells of the mouse aorta. British Journal of Pharmacology, v. 146, p. 288–295, 2005. UNNO, T. et al. Receptor signaling mechanisms underlying muscarinic agonistevoked contraction in guinea-pig ileal longitudinal smooth muscle. British Journal of Pharmacology, v. 139, p.337–350, 2003

04.019 Anti-inflammatory of the N-Methyl-Trans-4-Hydroxy-L-proline isolated from the leaves of Sideroxylon obtusifolium. Carvalho MAJ[1], Aquino PEA[1], Silveira ER[2], Costa RO[3], Souza AG[1], Lima KA[1], Cavalcante TMB[4], Viana GSB[1], Fonteles MMF[1] [1]UFC – Farmacologia, [2]UFC – Química, [3]UFC – Ciências Morfológicas e Fisiológicas, [4]UFC

Introduction: Sideroxylum obtusifolium (Humb. Ex. Roem. & Schult) T. B. Penn (Sapotaceae) of common occurrence in South America, is known in the Northeast of Brazil as "Quixabeira". The decoctions from the stem bark and leaves are used in folk medicine as anti-inflammatory. The present study evaluated by in vitro and in vivo models the anti-inflammatory effects of the compound N-methyl-trans-4-hydroxy-L-proline (NMP) isolated from the leaves of *Sideroxylon obtusifolium*. **Methods:** Male Swiss mice (25-30 g; n = 8-10) were used in the tests of paw edema and peritonitis, both induced by carrageenan.. Furthermore, the inflamed legs by carrageenan were collected and then sent for histological and immunohistochemical assay for TNF-α, iNOS, COX-2 and NF-kB. The participation of neutrophils was verified by myeloperoxidase dosage (MPO). **Results:** The results showed reduction of edema after treatment with NMP at all periods ($P < 0.05$). NMP decreased significantly the number of leukocytes infiltrate of the peritoneal cavity induced by carrageenan. The results of the in vitro tests showed reduced MPO activity ($P < 0.05$) but there was no antioxidant effect in DPPH test. There were significant reductions in the number of immunostained cells to TNF-α, iNOS, COX-2 and NF-kB in the groups treated with NMP ($P < 0.05$). **Conclusion:** The findings of this study indicate that NMP has significant anti-inflammatory effects that can be favorable as pharmacological tool for the treatment of conditions in which these pathways are highlighted. **Financial Support**: CNPq, CAPES e UFC. Animal Research Ethical Committee: 02/15

04.020 Nebulized gold nanoparticles down-regulates inflammation and lung remodeling in a murine model of steroid-resistant asthma via AKT suppression and HDAC2 activation. Serra MF[1], Pimentel A S[1], Cotias AC[1], Lanzetti M[1], Hickmann J[2], Arantes ACS[1], Silva PMR[1], Cordeiro RSB[1], Barreto E[2], Martins MA[1] [1]Fiocruz – Fisiologia e Farmacodinâmica, [2]UFAL

Introduction: The reduced responsiveness to anti-inflammatory effects of glucocorticoids (GCs) is a significant barrier to an effective therapeutic management of severe asthma. Gold-based compounds have a long history of therapeutic use for the treatment of chronic inflammation due to its anti-inflammatory and anti-oxidant properties. We recently reported that nasal-instilled gold nanoparticles (AuNPs) prevented central features of asthma in short-term models of this disease. Here, we sought to determine the effectiveness of aerosolized gold nanoparticles in a long-term murine model of steroid-resistant asthma. **Methods:** Mice of strain A/J were subcutaneously sensitized at days 0 and 14 by a suspension of $Al(OH)_3$ and ovalbumin (OVA), and challenged for 9 consecutive weeks, once a week, starting at day 19 post-sensitization. Three weeks after the beginning of OVA challenges, mice were subjected to daily interventional nebulizations (2.5 L/min, 30 min) of either 12 nm AuNPs (0.4 µg/mL) or budesonide (7.5 mg/mL) 1 h before challenge. Lung function, leukocyte infiltration, mucus exacerbation, extracellular matrix deposition, cytokine generation and oxidative stress were evaluated 24 h after the last challenge. Western blotting was used to investigate AKT and Histone deacetylase 2 (HDAC2) expression. (CEUA license # L-030/15). **Results:** We found that Ova-challenged mice developed marked AHR, lung eosinophil and neutrophil infiltrations, and increased peribronchial fibrosis and mucus production as compared to sham-challenged mice. All these changes were inhibited in mice treated with AuNPs, but not budesonide. Similarly, increased lung tissue levels of IL-4, IL-13, IL-17, eotaxin-1, eotaxin-2, KC and TARC appeared reduced after nebulized AuNPs, but remained unaltered following budesonide in this model. Furthermore, AuNPs treatment decreased the levels of TBARs (2,3±0,2 to 1,3±0,1 nMol/mg protein - Mean ± SEM, n=7) and restored catalase baseline levels. In contrast, budesonide did not interfere with TBARS or antioxidant enzyme activities. Finally, we observed a decreased expression of AKT (0,57± 0,10 to 0,24± 0,01 -Mean ± SEM, n=3) while HDAC-2 expression was up-regulated (0,51± 0,07 to 1,3± 0,11-Mean ± SEM, n=4) in mice treated with AuNPs, but not budesonide. **Conclusion:** We show that aerosolized AuNPs inhibits AHR, eosinophilic and neutrophilic lung inflammation, and airway remodeling and mucus exacerbation in a murine model of asthma, which expresses a marked refractoriness to glucocorticoid treatment. The protective effects of AuNPs treatment correlates anti-inflammatory properties and effectiveness in combating oxidative stress imbalance, in a mechanism probably related to up-regulation of HDAC2 and suppression of AKT expression. Taking together, these results suggest that AuNPs should be further investigated as a therapeutic alternative for controlling difficult-to-treat asthma. **Financial Support:** CNPq, FAPERJ and CAPES.

04.021 Refractoriness of macrophages from atypical chemokine receptor (ACKR)2 mice towards stimulation to LPS and silica particles *in vitro*. Correa AMC, Dias DF, Sá YAPJ, Ferreira TPT, Serra MF, Martins MA, Martins PMRS Fiocruz

Introduction: Silicosis is an occupational disease characterized by lung inflammation and chronic fibrosis, associated with granulomatous formation. Chemokines are principal regulators of leukocyte activation and migration, being considered as important mediators in inflammatory processes. We demonstrated that mice genetically modified for the atypical chemokine receptor (ACKR) 2 exhibited reduced of lung fibrosis and lung function decrease after intranasal silica provocation. **Aim**: This study was undertaken in order to investigate potential target cells involved in the refractoriness of ACKR2 knockout mice, mainly focusing on macrophages. **Methods**: Both sexes C57BL/6 ($ACKR2^{+/+}$) and ACKR2 knockout mice ($ACKR2^{-/-}$) were used. Total and differential leukocytes of peripheral blood and bone marrow were evaluated in Neubauer chamber and cytocentrifuged smears stained by May-Grunwald-Giemsa, respectively. For *in vitro* systems, peritoneal macrophages or bone marrow derived macrophages (BMDM), stimulated with lypopolissacharide (LPS – 0.1 and 0.5 µg/mL) or silica (12.5 and 125 µg/mL) particles, were used and the generation of cytokine/ chemokine being measured by ELISA. Markers used to identify classically (M1) and alternatively activated (M2) macrophages included TNF-α and CCL17/TARC, respectively. All procedures were approved by the Ethics Committee on Animal Use (CEUA) of Fiocruz in the LW57/14 license. **Results**: We noted similar levels of leukocytes were detected in the peripheral blood and bone marrow of $ACKR2^{-/-}$ as compared to those of $ACKR2^{+/+}$ mice. In another set of experiments, we showed that macrophages recovered from the peritoneal cavity of $ACKR2^{+/+}$ produced increased levels of TNF-α after stimulation with LPS (0.5 µg/mL) and silica (12.5 µg/mL), a response clearly attenuated in the case of macrophages from $ACKR2^{-/-}$. Additionally, we noted that BMDM polarized to M1-like or M2-like macrophages responded to stimulation to LPS (0.1 µg/mL) with an increase in the generation of TNF-α and CCL17/TARC. In line with previous data, macrophages from $ACKR2^{-/-}$ were significantly different from those from $ACKR2^{+/+}$ mice. **Conclusion**: Our findings show that there is no difference in total and differential leukocyte counts in peripheral blood and bone marrow of $ACKR2^{-/-}$ mice, as compared to those of $ACKR2^{+/+}$ animals. In contrast, the response of peritoneal and BMD macrophages recovered from $ACKR2^{-/-}$ animals to stimulation with LPS and silica particles *in vitro* was significantly lower, supporting the interpretation that the reduction in the lung response of ACKR2 knockout mice to silica provocation could be accounted for by down-regulation of macrophage function. Additional experiments are needed in order to clarify the mechanism underlying this phenomenon. **Financial support**: FIOCRUZ, CNPq, FAPERJ and CAPES.

04.022 Production and release of pro-inflammatory cytokines induced by agonists of different toll like receptors on glial satellite cells in vitro. Domingues LM, Lopes AH, Silva RL[2], Cunha TM – FMRP-USP – Farmacologia

Introduction: Satellite glial cells (SGCs) are located in the dorsal root ganglia (DRG) where form a sheath around the soma of the sensory neurons, thus they are able to modulate the neuronal activity. Induction of peripheral inflammation or nerve injury induces the production of pro-inflammatory cytokines and chemokines in DRG, but it is not clear which cells are responsible for this production and which mechanisms are involved in this process. SGCs express membrane receptors called toll-like receptors (TLRs), which are recognized triggers of the production of pro-inflammatory cytokines and chemokines. From these assumptions, the aim of this work was to evaluate the production/release of pro-inflammatory cytokines mediated by TLR2, TLR4 and TLR9 agonists on SGCs in vitro. **Methods:** SGCs were cultured from the dorsal root ganglia cells of C57BL/6 wild type (WT), $TLR2^{-/-}$, $TLR4^{-/-}$ or $TLR9^{-/-}$ mice. SGCs cultures were incubated with different TLR2, TLR4 and TLR9 agonists: LPS (agonist of TLR4), ODN-CPG (agonist of TLR9), Pam3CSK4 and PGN (agonists of TLR2). Quantification of cytokines TNF, CXCL1 and IL-6 in cell supernatant were performed using the ELISA method. **Results**: Stimulation of SGCs culture with PGN, Pam3CSK4, LPS and ODN-CPG induced the production/release of cytokines CXCL1 and IL-6 in a concentration dependent-manner, while the production/release of TNF was not significant. The production/release of CXCL1, IL-6 and TNF induced by Pam3CSK4 and PGN was not significant in SGCs obtained from $TLR2^{-/-}$ animals, but there were not affected when stimulated with LPS, comparing to SGCs from WT mice. In SGCs obtained from $TLR4^{-/-}$ animals, did not produced/released of cytokines CXCL1 and IL-6 when stimulated with LPS, but there was a significant production of CXCL1 and IL-6 with the stimulus by Pam3CSK4. In SGCs obtained from $TLR9^{-/-}$ animals, there was a reduction of the production/release of cytokines CXCL1 and IL-6 with the stimulus by ODN-CPG, but there were not affected when stimulated with LPS, Pam3CSK4 or PGN. **Conclusion:** Satellite cells express functional TLR2, TLR4 and TLR9 receptors which when active inducing the production of CXCL1 and IL-6, but do not induce the production of significant quantities of TNF. Thus, this study has the potential to contribute to the understanding of the molecular mechanisms of CXCL1 and IL-6 production in the DRG. **Financial Support**: Conselho Nacional de Desenvolvimento Científico e Tecnológico. Ethics Committee License: 089/2016.

04.023 Mechanism of anti-inflammatory action of *Croton campetris* essential oil and β-caryophyllene constituent – MAACCEOBCC. Oliveira-Tintino CDM[1], Pessoa RT[2], Fernandes MNM[2], Alcântara IS[2], Silva BAF[2], Oliveira MRC[2], Martins AOBPB[3], Menezes IRA[2] [1]UFPE – Antibióticos, [2]Urca – Química Biológica, [3]UFPE – Fisiologia e Farmacologia

Introduction: Inflammation is a natural process that aims to eliminate or neutralize antigen agent to the body. This process is divided into acute and chronic phases, depending on the duration and cellular and vascular processes involved. Natural substances have been used successfully with an anti-inflammatory effect. This study evaluates the anti-inflammatory effect of *Croton campestris* essential oil (EOCC) and β-caryophyllene as its major constituent. **Methods**: The inflammatory process is evaluated by the peritonitis model. This model evaluates leukocyte migration and vascular permeability. In this assay, the mice were treated with 0.9% saline (n = 6, negative control), 5 mg/kg dexamethasone (n = 6, positive control), essential oil EOCC 50, 100 and 200 mg/kg (n = 6) and their corresponding doses of β-caryophyllene present in EOCC 4.97, 9.94, 19.88, 39.76 mg/kg (n = 6) and the naive group (n = 6). After 1 hour of treatment, the animals received an intraperitoneal injection of 1% carrageenan. After 4 hours, the animals were euthanized by CO_2 inhalation, and 3 mL of ice-cold heparinized PBS were injected into the peritoneal cavity. The peritoneal lavage sample was read in cell count ABX Micros 60 (Horiba®) and the percentage of leukocytes, lymphocytes and monocytes was counted. Vascular permeability was measured by the Evans Blue protocol. In this method, 200 µl of the Evans Blue solution were administered intraocularly concomitantly with administration of 1% carrageenan. After 4 hours, the animals were euthanized and 3 mL of PBS were injected into the peritoneal cavity. The peritoneal lavage was centrifuged for 2 minutes at 6000 rpm, and the supernatant was read by 520 nm filter spectroscopy. **Results**: In the peritonitis test, the groups of dexamethasone (5 mg/kg), EOCC (100 mg/kg) and β-caryophyllene (19.88 mg/kg) reduced the percentage of lymphocytes in relation to the control in 56.41%, 42% and 28.34%, respectively. These same groups caused a significant reduction of the monocytes in relation to the control, being this reduction of 64.9%, 39.9% and 65.61%, respectively. In the vascular permeability test, these same groups had a respective absorbance value of 70.3%, 59.4% and 64.35% lower than the salt group. **Conclusion**: The essential oil (EOCC) and β-caryophyllene present a significant anti-inflammatory potential, interfering in the cellular pathway of the leukocyte migration process and vessel permeability. **Financial Support**: CNPq, Capes, FUNCAP 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.

04.024 A late component of neurogenic inflammation in the long-lasting orofacial model of oedema in rats. Queiroz BFG[1], Almeida MP[1], Francischi JN[1] [1]UFMG – Farmacologia

Introduction: We have shown previously that, differently from the paws, the rat cheeks show a long-lasting oedematogenic response to carrageenan (Frade et al., 2016). As the orofacial tissue is extensively innervated by the trigeminal nerve, we have assessed the contribution of endogenous neuropeptides released from this mainly sensory nerve, to cheek oedema induced by carrageenan. **Methods:** Neonate Wistar rats (2-3 days of birth; N=6) under isoflurane anesthesia were injected subcutaneously in the dorsal region with capsaicin (CPS; 10 mg/ml; 50 □l) prepared as described (Nagy & Van Der Kooy, 1983). Control animals (N=6) were injected with the capsaicin vehicle (Veh; ethanol+tween 85+saline; 0.0015:0.0075:0.99v/v). One month later, when the animals weighed 150-200g, the right-hand cheek was injected with carrageenan (CG; 500□g) or substance P (5□g) in 100µl saline and the contralateral cheek received only 100µl saline. Cheek oedema was measured as the difference in thickness (in mm) between right and left cheeks, with a digital caliper (Mitutoyo, Japan) before (time 0) up to 144h, following agonist injections. **Results:** Capsaicin, one of the main tools to promote neuropeptide release, partially reduced the long-lasting cheek oedema induced by carrageenan in the rat cheeks, particularly on the second (CAPxCG=1.56± 0.20 or Veh=2.38±0.18) and third days (CAPxCG=1.07± 0.16 or Veh=2.05± 0.20mm) of the established response. A similar capsaicin treatment increased the cheek oedema induced by exogenous substance P administration, indicating local nerve desensitization. **Conclusion:** There may be a component of neurogenic inflammation in the late phase of the long-lasting oedematogenic response to carrageenan in the orofacial tissue of rats. References**:** 1) Frade TIC, Tissue-selective inflammation in the oral cavity of the rat. Inflammopharmacology 24:145–153, 2016; 2) Nagy JI, Effects of neonatal capsaicin treatment on nociceptive thresholds in the rat. J Neurosci 3:1145-1150, 1983. **Financial Support**: CNPq and FAPEMIG Experimental procedures were approved by the UFMG Ethics Committee for Use of Animals (Protocols n. 97/2013 and 368/2014).

04.025 Succinate receptor GPR91 is critical for development of experimental psoriasis. Norbiato TS, Veras FP, Melo B, Saraiva A, Ryffel B, Cunha TM, Cunha FQ, Alves-Filho JC FMRP-USP

Introduction: Psoriasis (PsO) is an inflammatory disease of the skin that affects 2-5% of world population. The etiology of PsO is complex and is not completely elucidated. It is known that accelerated proliferation and early maturation of keratinocytes are responsible for the clinical manifestations of the disease, such as thickening of the epidermis and the appearance of psoriatic plaques. However, the mechanism of the pathogenesis remains unclear. Succinate is a metabolic intermediate of citric acid cycle, an important step of cellular metabolism. In immune system, the succinate receptor (GPR91) is mainly expressed in dendritic cells and macrophages and its activation promotes cells migration and cytokine production. Considering the immunomodulatory effects of GPR91 signaling, the aim of this study is to evaluate the role of this receptor in experimental psoriasis. **Methods and Results:** To investigate the role of GPR91 in the development of psoriasis, psoriasis-like skin inflammation was induced by topical application of imiquimod (IMQ) on the skin of GPR91 knockout ($GPR91^{-/-}$) mice. After the psoriasis induction, we found an increased expression of GPR91 in back skin of mice bys performing immunofluorescence assay. Notably, we observed strongly reduction of psoriasis-like skin inflammation score, pro-inflammatory cytokine levels determined by ELISA and expression of markers of keratinocyte hyperproliferation and activation (Lcn2, Keratin-17, S100A9) evaluated by qPCR in the back skin of the $GPR91^{-/-}$ mice in compared with WT mice. Moreover, H&E staining from skin sections showed reduced acanthosis, leukocytes infiltration in mice lacking GPR91 when compared with the control group. We then evaluated the population of IL-17-producing T cells ($IL17^{+}CD4^{+}$ or $IL17^{+}TCR\gamma\delta^{+}$) in the draining lymph nodes by flow cytometry after psoriasis induction. We found a reduced frequency of IL-17-producing T cells in GPR91 knockout mice. Finally, we evaluated the population of Langerhans cells ($EpCAM^{+}CD11c^{+}$) in the skin and draining lymph nodes of mice. We found reduced frequency and number of Langerhans in GPR91 knockout mice and reduced expression of IL-23 in the skin. **Conclusion:** Taken together, our results demonstrate that GPR91 receptor is important to development of the experimental psoriasis by enhancing the production of IL-23 by Langerhans cells and, consequently, controlling the differentiation of IL-17-producing T cells.

04.026 The alarmin S100A9: A key target for treatment of psoriasis .Melo B[1], Protasio F[1], Prado D[1], Costa L[2], Souza C[2], Lima D[3], Nakaya H[3], Cunha T[1], Cunha F[1], Alves-Filho JC[1] [1]FMRP-USP – Farmacologia, [2]FMRP-USP, [3]FCF-USP – Análises Clínicas e Toxicológicas

Introduction: Psoriasis (Ps) is an immune-mediated chronic inflammatory skin disease, characterized by accentuated proliferation and abnormal differentiation of keratinocytes and infiltration of inflammatory cells in the dermis. S100A9 is an alarmin that is produced by keratinocytes and myeloid cells in inflammatory conditions. However, the role of this molecule in the development and maintenance of the inflammatory response in Ps remains not well understood. Herein, we investigated the role of S100A9 in the development of psoriasis. **Methods and Results**: Bioinformatical analysis of an online database containing human gene expression information showed that the s100a9 is overexpressed in lesional skin from Ps patients. These data were confirmed by immunofluorescence and western blot that showed an overexpression of s100a9 in the lesional skin from Ps patients compared with paired samples of nonlesional psoriatic skin. These levels of s100a9 were positively correlated with the expression of keratin-17, a keratinocyte activation marker. To investigate the role of S100A9 in the development of Ps, psoriasis-like skin inflammation was induced by topical application of imiquimod (IMQ) on the back skin of S100A9-deficient mice ($S100A9^{-/-}$) or paquinimod (10mg/kg, v.o) pretreated mice. IMQ exposure induced *s100a9* mRNA and S100A9 protein expression in a rapid and time-dependent manner in the skin and lymph node of mice and remained elevated until the end of the experiment (6^{th} day). Notably, inflammation, assessed by epidermal thickness measurement and H&E-stained histological sections, was significantly reduced in $S100A9^{-/-}$ or paquinimod treated-mice compared with wild-type (WT) control mice. To determine which S100A9-producing cell contributes to the Ps development we performed a chimera and showed that both keratinocytes and myeloid cells are important for the production of s100a9 and contribute to the development of psoriasis. Moreover, the expression of *IL-23,* in the skin, was reduced, which might explain the reduction of IL-17-producing gamma-delta T cells in the lymph nodes of $S100A9^{-/-}$ or paquinimod-treated mice. **Conclusion**: We showed that the alarmin S100a9 plays an important role in the development of psoriasis. Thus, targeting S100A9 could be a future strategy for pharmacological treatment of psoriasis and this protein can be used as a marker of disease activity.

04.027 Influence of cyclooxygenase-2-derived prostaglandins on skeletal muscle degeneration and regeneration events. Zuntini ACS[1], Damico MV[1], Fortes-Dias CL[2], Spadacci-Morena DD[3], Moreira V[1] [1]Unifesp-EPM – Farmacologia e Inflamação, [2]Fundação Ezequiel Dias, [3]IBu – Fisiopatologia

Introduction: Skeletal muscle injuries are common in myodegenerative diseases and sports, and the investigation about regulatory mechanisms of tissue regeneration is important for the development of novel reparative therapies. Usually, the phases of skeletal muscle degeneration and regeneration are accompanied by inflammatory response, which mediator profiles are important for the successful of tissue repair. Although some studies in vitro have demonstrated the cellular proliferative activity by prostaglandins (PGs) produced from cyclooxygenase (COX) pathways, the regulatory role of PGs synthesized from COX-2 pathway on muscle degeneration and regeneration in vivo, is still unclear. The aim of this study was to analyze the regulatory role of COX-2-derived PGs on morphological events observed during skeletal muscle degeneration and regeneration induced by a myotoxin. **Methods:** Male Swiss mice (20 g) received intramuscular (i.m) injection of phospholipase subunit (CB) of the crotoxin complex, isolated from Crotalus durissus terrificus snake venom (7,5 µg/animal/ 50µL) or saline solution. After 30 min, distinct groups of animals received lumiracoxib (p.o), a selective COX-2 inhibitor (20 mg/kg/ 200µL), or vehicle (1%Tween-80). After 6 and 24 h, 3, 7, 14 and 21 days (d) of i.m injection, the animals were euthanized and the right gastrocnemius muscle was dissected and fixed in 4% paraformaldehyde solution. For histological analysis, non-consecutive sections (4 µm) of muscle tissue were stained with hematoxylin/eosin, and images from 4 distinct fields were captured and scanned for histological analysis. The muscular degeneration phase (6 and 24h, 3d) was characterized by the percentage of myofiber lesions and by counting the cellular influx to the lesion site. The regeneration phase (7, 14 and 21d) was characterized by the percentage of central nuclei in relation to total myofibers. **Results:** Considering the degeneration phase, animals that received CB (i.m.) and lumiracoxib (p.o.) presented significant decrease ($p<0.001$) in fiber lesions (28,5□6,7%-24h; 14,02□4,4%-3d) when compared to those treated with CB and vehicle (46,8□2,2%: 24 h; 34,43□7,7%: 3 d). Regarding the cellular infiltrate, animals treated with CB and lumiracoxib showed statistically lower cell counts ($p<0.001$) (1669,3□254,1-24h; 819,7□130,1%-3d) when compared to those that received CB and vehicle (3431,3□92,4:-24h; 1991,3□339,7%-3d). Morphological analysis of regeneration phase (7 to 21d) showed that in the group treated with CB and lumiracoxib, the central nuclei percentage (around 32,15□2,4% to 38,70□6,7%) was not different ($p<0.05$) to the percentage of mice treated with CB and vehicle only (36,12□10,3% e 46,35□8,8%). **Conclusion:** These data suggest that COX-2-derived PGs up-regulate skeletal muscle degeneration by inducing cellular influx and mechanisms that contribute to myofiber lesion. In contrast, considering the morphological aspect, the results suggest that this group of lipid mediators does not exert any regulatory influence in regenerative phase of skeletal muscle after injury. **Financial Support**: FAPESP. Ethics Committee in Animal Experimentation of UNIFESP (CEUA 3051010816).

04.028 Mast cells depletion improves bone markers expression on mandible of spontaneously hypertensive rats with periodontal disease. Brito VGB[1,2], Barreto AEA[1,2], Patrocinio MS[1], Sousa MCL[1], Beltran CT[1], Queiroz DP[1,2], Vieira LV[1,2], Lara V[3], Santos CF[4], Oliveira SHP[1,2] [1]FOA-Unesp – Ciências Básicas, [2]FOA-Unesp-SBFis, [3]FOB-USP – Ciências Biológicas, [4]FOB-USP – Estomatologia

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 lead to exacerbated inflammatory response and enhanced bone resorption. Resident immune cells have a crucial role in PD progression; however, mast cells participation is not yet well comprehended. **Objectives:** We aimed to evaluate the role of mast cells on gene expression of bone metabolism on mandibles of normotensive and hypertensive rats with experimentally induced PD. **Methods:** 10 weeks old male Wistar and SHR were pre-treated with compound 48/80 (mast cell degranulation-inducing pharmacological agent) and subjected to 15 days of PD, induced by a bilateral silk wire ligature placed on the first inferior molars. Experimental groups were labeled as Control (C), PD and 48/80+PD of Wistar (W) and SHR (S) animals. Hemi mandibles were harvested for real-time RT-PCR analysis of bone formation markers (Runx2, Osterix, β-catenin, osteoprotegerin, BMP-2, alkaline phosphatase, osteocalcin, osteopontin and bone sialoprotein) and bone resorption/remodeling markers (tartrate-resistant acid phosphatase, RANK, RANKL, cathepsin K, MMP-2 and -9 and OSCAR). The protocol was approved by Institutional Animal Care and Use Committees (School of Dentistry of Araçatuba; Process 00686-2016. **Results:** Gene expression analysis of bone formation markers didn't reveal major alterations, except for osteopontin, which was significantly increased in WPD and SPD groups. However, the bone resorption markers TRAP, MMP9, CtsK and, Oscar, showed higher expression on SC, compared to WC. PD groups showed higher expression of those markers compared to C group, especially on SPD, who had further increased resorption markers expression. Mast cells depletion previous to PD induction were able to reduce those resorption markers, and interestingly, it was more significant on hypertensive animals (S+48/80+PD). **Conclusion:** Our results indicate that mast cell may have an important role in bone resorption markers expression on hypertensive animals with PD, with possible participation in alveolar destruction. These are preliminary results, and other experimental analyses are currently been executed to find further evidence for our better understanding of this effects. **Acknowledgment:** FAPESP (Grant #2015/03965-2) and CAPES, for Financial Support.

04.029 Dimethylfumarate: Comprising its harmful effects in topical application. Prudente AS, Lückemeyer DD, Ferreira AM, Macedo Juniro SJ, Ferreira J UFSC – Farmacologia

Introduction: Dimethylfumarate (DMF) has also been a systemic therapeutic option in the treatment of moderate to severe psoriasis (FUMADERM ®) since 1994 and has recently been approved as a disease modifying drug for patients with multiple sclerosis (MS) through a systemic use (via oral) of Tecfidera® (Europe, Australia, USA and Canada), being favorable because its oral bioavailability probably leads to improvement in treatment adherence. However, between 2007 and 2008, in northern Europe and England, a small epidemic, showing severe cases of contact dermatitis in the back and buttocks related to sofas and armchairs, newly acquired by the patients, originating in China. Later, a relationship was established between this dermatitis and the DMF, after the chromatographic isolation of this allergen that was found in small bags inside this furniture to control their humidity. Subsequently, it was confirmed by epicutaneous tests when these patients reacted positively to DMF. DMF is a fumaric acid ester having an α, β-unsaturated group structurally similar to TRPA1 receptor activators, the aim of this work was to investigate the role of TRPA1 in animal model of contact dermatitis caused by DMF. Methodology: C57BL-6 mice (20-25 g, N = 6) were used and ear edema was induced by topical application. Thickness (μm) of the ear was measured before and 4 h after application of different treatments using a digital micrometer (Mitutoyo® S-293). All the treatments were dissolved in 20 μl of acetone and applied on the inner ear of mice. The Ethics Committee on Animal Use of UFSC (PP00872) approved experiments. **Results:** The topical application of DMF in the ear of the mice was able to cause an increase in the ear thickness of the animals in a dose and time dependent manner, with a peak at 4 hours and a maximum concentration of 10% as well as the increase of neutrophil migration significantly. Similar to others allergens, DMF was able to cause a sensitization as evidenced by increased edema after a re-exposure. TRPA1 knockout mice prevented the formation of edema promoted by DMF in 86.6 ± 4.5%, this response was also reduced by the use of agonists (HC-030031 and A-0967079) and ablation of the TRPV1-positive fibers by resineferatoxin, both in the acute response and in the re-exposure. The use of a partial agonist of hydroxycarboxylic acid receptor 2 (HCA2), No-steroidal anti-inflammatory (NSAID) together with DMF promotes a reduction of edema caused by DMF only. **Conclusion:** The results obtained in the present work suggest that the irrigative action caused by the DMF is due to its action in TRPA1 directly or indirectly through the HCA2, by interacting with prostaglandins pathway. **Support**: CNPq

04.030 Modeling acute exacerbation of chronic obstructive pulmonary disease by combining cigarette smoke inhalation and H1N1 infection in mice. Ferrero MR[1], Ferreira TPT[1], Torres J[1], Bento S[1], Arantes AC[1], Coutinho D[1], Garcia CC[2], Martins MA[1]
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Introduction: Chronic obstructive pulmonary disease (COPD) highly reduces the life quality of people and can be fatal. Infections strongly exacerbate symptoms and severity of COPD (AECOPD), and massive neutrophil infiltration into the lung tissue is a pivotal underlying component. Since the development of animal models reproducing more properly AECOPD will be helpful in the identification of associated mechanisms and novel therapies, we studied here whether H1N1 virus would exacerbate cigarette smoke (CS)-induced lung inflammation in C57Bl/6 mice. **Methods:** Forty-two female C57Bl/6 mice were equally distributed into 6 groups (n=7) as following: (1) ambient air (AA), (2) AA + 100 pfu (AA100), (3) AA + 1000 pfu (AA1000), (4) cigarette smoke (CS), (5) CS + 100 pfu (CS100) and (6) CS + 1000 pfu (CS1000). Mice were exposed to CS 7 days per week for 12 days and H1N1 infections with 100 or 1000 pfu were performed at day 7 with A/PR/8/34 strain intranasally. Weight loss, lung mechanics, as well as inflammatory and oxidative/anti-oxidative changes in lung tissue were assessed 24 h after the last exposure to CS. In other set of experiments, we evaluated our model response to 1 mg/Kg dexamethasone treatment in AA1000 and CS1000 groups of mice analyzing the above-mentioned parameters as also mortality. **Results:** We found that only mice infected with 1000 pfu, exposed or not to CS, lost weight significantly. Levels of neutrophil accumulation in BAL fluid, as well as lung MPO, TNF-α, IL-6, KC, MIP-1-α and MCP-1 appeared significantly exacerbated in CS1000 but not in CS100 group when compared to AA100 and AA1000. In CS group of mice, none of those parameters was altered. Viral infection alone decreased catalase activity while augmented glutathione peroxidase activity and lipid peroxidation in a load dependent way. CS exposure exacerbated lipid peroxidation and glutathione peroxidase activity in the CS100 group only, but had no effect in catalase activity. Survival experiment showed that CS exposure exacerbated H1N1 induced lethality (37.5% in AA1000 versus 75% in CS1000). Dexamethasone treatment in CS1000 mice reduced mononuclear cell infiltration but could not prevent neutrophil exacerbated accumulation in BAL neither decrease MPO activity in lung tissue. Accordingly, dexamethasone treatment could not prevent the increased mortality rate of CS1000 group of mice. **Conclusion.** Our results show that combination of CS and H1N1 infection led to synergistic exacerbation of pivotal lung inflammatory changes in C57Bl/6 mice including increased neutrophilic activity, which was clearly non-responsive to glucocorticoid treatment. This short-term AECOPD model may be suitable for investigation of mechanisms and putative therapies relevant in COPD exacerbations. **Financial support:** INCT-INOFAR, CNPq and FAPERJ. CEUA – L030/15, L048/16.

04.031 Characterization of model equivalent to sunburn induced by Ultraviolet B radiation in skin of *Hairless* mice. Freitas KM[1], Barcelos LS[2], Caliari MV[3], Lopes MTP[4] [1]UFOP – PPGBIOTEC/NUPEB, [2]ICB-UFMG – Biofísica e Fisiologia, [3]ICB-UFMG – Patologia, [4]ICB-UFMG – Biofísica e Farmacologia

Introduction: UVB radiation exposure is considered an important risk factor responsible for skin disorders, such as excessive free radical production, antioxidant system depletion and inflammation (FILIP et al., 2011; CHOI et al., 2014). Currently, models of acute exposure to UVB are developed to identify, evaluate and prioritize chemical agents and natural products as to the ability to prevent damage caused by UV and, consequently, carcinogenesis (STEELE & LUBET, 2010). We proposed to characterize an experimental model of single dose UVB irradiation with the objective of producing a lesion in mice similar to sunburn. **Methods**: *Hairless* mice (n=49) were irradiated by UVB light (dose 2.4J/cm^2 - Coler-Parmer®, 15W, maximum length of 312 nm) in dorsal area. The mice groups (0-168 h) were observed macroscopically, imaged and killed and the full thickness of the dorsal skins were removed for further analysis. The levels of inflammatory infiltrate (neutrophils, myeloperoxidade – MPO), nitrite, superoxide dismutase (SOD) activity and colagen levels were determined by kinetic-colorimetric assay. The catalase activity was measured spectrophotometrically and GSH (Glutathione) determined by fluorescence assay. In addition, the production of cytokines was measured by ELISA.Tissue sections (5 µm) were processed for microscopic studies and stained with hematoxylin and eosin (H&E). **Results**: Compared with the intact status of the control (*sham*), the UVB groups showed pathologic alterations in physiologic properties and tissue integrity. The skin presented, macroscopic analysis, showed marked erythema and edema even 12 h and 24 h after UVB, respectively. After 72 h, we observed the pigmented skin and the brown color, more rough appearance.The inflammatory infiltrate was observed by MPO activity from 48 h and significant from 72 h (5.0 fold, $p < 0.05$). In fact, histological analysis showed predominant infiltration of intense neutrophils in particular 48 h. As regards proinflammatory cytokines, the data showed a significant increase in TNF-α levels after 12 h (3.7 fold, $p< 0.001$). The profile of this cytokine was also altered after 72 (3.5 fold, $p< 0.05$ and 120 h (2.5 fold, $p < 0.05$) after irradiation, as well VEGF levels (2.8 and 4.8 fold $p< 0.05$, respectively). Both cytokines returned to baseline in time 168 hs. UVB promoted significant reduction of nitrite levels 6 (3.8 fold, $p< 0.05$) e 12 h (15.4 fold, $p< 0.05$), followed by significant increase (2.3 fold, $p< 0.05$) after 24 h of radiation. In addition, radiation stimulated the antioxidant system by significantly increased SOD activity after 12h (2.0 fold, $p<0,01$), and we observed significant increase in CAT activity in 24 h (2.5 fold, $p<0,01$). On the other hand, GSH levels showed reduced from 6 h (3.2-fold, $p <0.01$) and returned to the baseline from 72 h after exposure to UVB. **Conclusion:** Together, our results demonstrate the potential of this cutaneous model (sunburn) as a tool to study various aspects of acute skin responses to the marked damage of UVB radiation. **Reference:** CHOI, K. S. ***Arch Biochem Biophys***, v. 559, p. 38. 2014. FILIP, A. ***Journal of Medicinal Food***, v.14, p. 761. 2011 STEELE, V. E. ***Seminars in Oncology***, v.37, p.327. 2010 **Financial support.** CNPq, FAPEMIG e CAPES. Protocol CETEA 174/2010.

04.032 *Dilodendron bipinnatum* extract ameliorates TNBS-induced colitis in rats by inhibiting TNF-α, supporting mucus production and promoting antioxidant effect. Oliveira RG[2,1], Ferreira LA[2], Miyajima F[3], Pavan E[1], Damazo AS[1], Martins DTO[1]
[1]UFMT – Basic Sciences in Health, Faculty of Medicine, [2]UNIC – Pharmacy, [3]UFC – Neuropharmacology, Drug Research and Development

Introduction: *Dilodendron bipinnatum* (Db), Sapindaceae, is a tree naturally occurring in Brazil's Pantanal basin and whose decoction and maceration of its stem bark yields an extract popularly used for the treatment of inflammation. Our recent *in vivo* studies have demonstrated that the hydroethanolic extract of inner stem bark of Db (HEDb) inhibited leukocyte migration and the levels of TNF-α and IL-1β in a murine model of LPS-induced peritonitis, whilst at the same time increased the concentration of the IL-10. Ulcerative colitis (UC) is a persistent inflammatory condition of unclear etiology affecting the large bowel. Its pathophysiology presents with a dysregulation of the normal immune system driven by an antigenic trigger, thus leading to a prolonged mucosal inflammatory response. **Aims:** We sought to investigate the pharmacological potential and immunological regulatory activity of HEDb in an experimental rat model of UC. **Methods:** Each group of rats (n=8) was pretreated with, either a vehicle (Veic, 0.9 % saline), HEDb (20, 100 or 500 mg/kg), or mesalamine (Mesa-500 mg/kg) as standard drug, at 48, 24 and 1 h prior to the administration of TNBS/50% ethanol, and at 24 h after UC induction. The plausible mode of action of the HEDb was assessed using damage score macroscopic and by measuring MPO and GSH antioxidant activities. Additionally, the histopathological analyses of the UC by H&E and PAS staining were conducted alongside the quantification of the pro-inflammatory cytokine TNF-α by ELISA. **Results:** Compared to the Veic, a significant reduction in macroscopic damage scores was observed for all conditions tested with HEDb. Similarly, the HEDb at the highest dose reduced the MPO activity by 48% (p<0.001) when compared to the colitis induced group (3.80 U/mg tissue), while Mesa reduced it by 69% (p<0.001). The Veic group had a 40% reduction (p<0.001) in GSH levels in the colon compared with the sham group (474 µg/g tissue). Interestingly, GSH levels were restored by 58% and 70% (p<0.001) with the conditional treatment with HEDb at doses of 100 and 500 mg/kg respectively, whereas Mesa restored it by 123% (p<0.001) compared to the Veic. As expected, colonic injury by acute TNBS administration was characterized by a 3-fold increase in the levels of TNF-α compared to sham (p<0.001). In contrast, HEDb at 500 mg/kg and Mesa both resulted in a reduction in the levels of TNF-α response by 72% and 64%, respectively, when compared to the Veic (26.5 ± 3.82 pg/mg of protein). The HEDb (100 and 500 mg/kg) accounted for a significant decrease in hemorrhagic damage, leukocytes infiltration, edema and increasing mucus compared to the Veic group. HEDb at the two highest doses also reestablished the mucus production by approximately 40%, which was comparable to the Mesa**.** **Conclusion:** Our data were consistent with the histological findings, in which HEDb reduced the gross lesions and exerted a protective action in the colon by supporting mucus production and antioxidant effect. **Financial Support and Acknowledgments:** INAU-INCT/CNPq, FAPEMAT, CAPES/Pró-Amazônia and UFMT.

04.033 Corticosterone and melatonin crosstalk in controlling inflammatory processes in toads (***Rhinella ictérica***). Bastos PR[1], Cruz-Machado SS[1], Markus RP[1], Gomes FR[1], Ferreira ZS[1] [1]IB-USP – Fisiologia

Introduction: Several stressors, including pollution and pathogens, have contributed to amphibian population decline/extinction (Daszak et al, Emerg. Infect. Dis. 5:735, 1999). In mammals, a dual effect of corticosterone (CORT) on plasma melatonin (MEL) content has been observed dependent on circulating CORT levels (Ferreira et al, J Pineal Res. 38:182, 2005; Fernandes et al, J Pineal Res. 41:344, 2006). Nocturnal MEL surge is also modulated by inflammatory agents (da Silveira Cruz-Machado et al, J. Pineal Res. 49:183, 2010; Carvalho-Souza et al, Front in Endocrinol 2:1, 2011) and participates in inflammatory responses as it is suppressed at the beginning of an inflammatory response in order to allow a full mounting of an innate immune response (Markus et al, Neuroimmunomodulation, 14:126, 2007). Once pineal MEL synthesis is inhibited, a shift to an autocrine/paracrine production by immune cells is observed, a concept designed Immune-pineal Axis (Markus et al, Int J Mol Sci. 14:10979, 2013). Recently, the dual CORT effect on plasma MEL content was also observed on the levels of ocular MEL in anurans after ACTH treatment (Barsotti et al, Comparative Biochemistry and Physiology, Part A 204:177, 2017). Our aim was to investigate the effects of LPS-induced systemic inflammation on the production of mediators that participate in the assembly and resolution of inflammatory responses in amphibians. **Methods:** Adult males collected in January 2015 (São Luiz do Paraitinga, SP, Brazil (IBAMA licence 29896) were kept individually in plastic boxes with air circulation, 22±2°C, LD 12:12h, free access to water, fed once a week with crickets and cockroaches. CORT and MEL daily variation were determined in blood collected at the 3h interval through 24h via cardiac puncture. In another protocol, the animals were injected into the dorsal lymph sac with saline or LPS (from *Escherichia coli*, serotype 0127:B8; 2.0mg/Kg; ZT15) and euthanatized at ZT17. Blood was collected for measuring CORT, MEL and cytokines, while the eyes were removed for determining an extra-pineal content of MEL. MEL and CORT were measured by ELISA Kit and the cytokines by Cytokine/Chemokine Magnetic Bead Panel (RECYTMAG-65K11 MILLIPLEX MAP; CMC-SF, IL1β, IL2, IL4, IL6, MCP1, IL10, IL12p70, IL17, IL17A, IFN ϒ, TNF). **Results:** The temporal pattern of CORT release was evidenced by increased levels in the dark period, peaking at the transition in ZT12 (15.4±3.8 pg/ml; n=3). The nocturnal temporal pattern of MEL production was evidenced which peaks at ZT18 (80.2±23.4 pg/ml; n=3). LPS-induced systemic inflammation led to a 10-fold increase in CORT (saline: 9.0±1.1 ng/ml, n=7; LPS: 101.7±33.8 ng/ml, n=6; $p<0.05$) while a decrease in plasma MEL (saline: 58.5±10.4 pg/ml; LPS: (14.5±6.6 pg/ml, n=6; $p<0.05$). No effect was observed on the ocular MEL levels (saline: 41.0±7.1 pg/ml; LPS: 30.5±2.4 pg/ml, n=7). LPS-induced systemic inflammation up-regulated the cytokine IL1β (saline: 14.6±6.3 pg/ml; LPS: 37.4±1.2 pg/ml, n=3). The cytokines IL10, IL12p70, IL17, IL17A, IFN ϒ, TNF were non-detectable while the remaining were unregulated. **Conclusion:** Our data demonstrate a circadian variation on CORT and MEL production not yet demonstrated in *Rhinella icterica,* as well as the existence of a bidirectional regulatory mechanism between the immune and the endocrine systems in amphibians, pointing to the presence of an active Immune-pineal Axis in the stress responses in amphibians. Support: CAPES, FAPESP, CNPq. (CEUA/IB 262/2016).

04.034 Melatonin (MEL) synthesis by pineal gland is regulated by fungi and bacterial infectionMelatonin (MEL) synthesis by pineal gland is regulated by fungi and bacterial infection. Silva-Souza E, Cruz-Machado SS, Markus RP IB-USP – Fisiologia

MEL rise in the circulation and cerebrospinal fluid is a neuroendocrine response of the pineal gland to adjust endogenous rhythms to environmental lighting. The pineal gland, composed of pinealocytes and glia cells, is out of the blood-brain barrier, senses acute inflammatory responses, which blocks nocturnal MEL production due to activation of toll-like receptor 4 (TLR4), which leads to inhibition of the transcription of *Aanat* (aralakyl-N-acetyltransferase) (Markus et al., Int J Mol Sci, 2013). This enzyme catalyzes the conversion of serotonin to N-acetylserotonin, the precursor of melatonin. The rat pineal gland expresses other six coding genes for TLRs (*Tlr1*, *Tlr2*, *Tlr3*, *Tlr6*, *Tlr7* and *Tlr9*) (da Silveira Cruz-Machado et al., Sci Rep, 2017). Gram-negative bacteria pathogen-associated molecular pattern, lipopolysaccharide (LPS), inhibits the nocturnal MEL rise (Tamura et al., PloS One, 2010), whilst, *Leishmania amazonensis* that interacts with TLRs through lipophosphoglycan (Tuon et al., Infect Immun, 2008), does not alter the circadian rhythm of MEL (Laranjeira-Silva et al., J Pineal Res, 2015). Given that suppression of nocturnal melatonin peak is essential for migration of leukocytes to infected sites, we evaluated whether the pineal gland is a target for fungal and Gram-positive bacterial infection. **Methods:** Adult male Wistar rats (8-10 week-old) were euthanized by decapitation. Cultured pineal glands (3 days) were treated with Pam3CSK4, a synthetic agonist of TLR1 and TLR2 that mimics lipoprotein of gram-positive bacteria, or by zymosan, a ligand found on the surface of fungi that binds to TLR2 or TLR6 (1.0 µg/mL, 5-60 min). Expression of TLR2 and TLR6 was evaluated by immunocytochemistry in isolated pinealocytes. Nuclear translocation of NFkappaB was determined by EMSA. Noradrenaline-induced MEL synthesis in cultured glands was quantified in the culture supernatant by HPLC. Institutional ethical committee (CEUA/IB 115/2010) approved this project. **Results:** Pinealocytes express both TLR2 and TLR6 receptors. Incubation of rat pineal glands with Pam3CSK4 or zymosan triggers activation of p50 and p65 NFkappaB proteins. The analysis of the time-dependent effects suggests a differential pattern of regulation of NFkappaB: Pam3CSK4 increases nuclear translocation after 15 min, which remained active up to 60 minutes. As for zymosan, the effects were transient as the increase after 15 min was decreased after 30 minutes. Pam3CSK4 and zymosan also inhibited noradrenaline-induced MEL synthesis in cultured glands by 48% or 58%, respectively (control 29.4 ± 3.2 ng/well; N=6 to 8 glands). Conclusions: Detection of fungal or bacterial infection by TLR2 and TLR6 expressed in pinealocytes is translated by activation of NFκB and reduction of MEL production. Thus, we provide new findings to support a model where pinealocytes act as a sensor and integrator of signals from the environment and immune system. Understanding the mechanisms of the immune-pineal axis activation provides a pharmacological target for the pathological deviations that can be caused by infectious diseases.

04.035 A novel monocyte subset contributes to clearance of damage tissue during sterile inflammation and bacterial infection in the liver. Dal-Secco D[1], Jenne CN[2], Kolaczkowska E[2], Wong CHY[2], Petri B[2], Ransohoff RM[3], Charo IF[4], Kubes P[2] [1]UFSC – Farmacologia, [2]University of Calgary, [3]Lerner Research Institute, [4]University of California

Introduction: Monocytes are recruited from the blood to inflammation site, where they contribute to bacterial clearance, wound healing and tissue repair. There are at least two subsets of monocytes: classical or proinflammatory ($CCR2^{hi}CX_3CR1^{low}$) and nonclassical, patrolling, or alternative ($CCR2^{low}CX_3CR1^{hi}$) monocytes. A previous study from our group using a murine model of focal hepatic necrosis induced by localized thermal injury has shown that by a mechanism dependent on CCR2, the $CCR2^{hi}$CX3CR1^{low} monocytes were recruited early and persisted for at least 48 h, forming a ring like structure around the injured area. However, it is presently unknown whether pro-inflammatory, as well, anti-inflammatory monocytes subsets also are recruited to the liver at later time-points after bacterial infection. Therefore, we have evaluated the dynamic of different sort of monocyte accumulation in the *Staphylococcus aureus* infection-induced liver injury model from transgenic mice at later time points. **Methods and Results:** By using spinning disk confocal intravital microscopy and mice with fluorescent reporters for each of these subsets, we were able to track the dynamic spectrum of monocytes that enter a hepatic injury site in vivo. We observed that the $CCR2^{hi}$CX3CR1^{low} monocytes (75-90%±0.4/area of injury) were recruited early and persisted for at least 48 h, into the necrotic area. These monocytes transitioned, in situ, from $CCR2^{hi}$CX3CR1^{low} to CX3CR1$^{hi}CCR2^{low}$ in the bacterial injury site. This phenotypic conversion (88%±0.2/area of injury) was essential for optimal repair. Moreover, lack of CCR2 impairs (80%±0.3/area of liver) healing of the necrotic lesions *S. aureus*-induced at 72 h after infection. The collagen deposition was lower in CCR2 deficient mice (85%±0.5/area of liver). **Discussion:** Importantly, our data demonstrate a continuum of monocyte phenotypes rather than the two circumscribed profiles originally proposed. It was investigated in detail we do see similar to sterile injury responses in a preliminary series of data in an *S. aureus* model of liver infection. The responses to sterile injury and infection have been driven by millions of years of evolution and interestingly, both converge on a similar pathway, dependent on CCR2. This convergence likely reflects an optimal immune and repair response. Our new understanding of monocyte plasticity within the tissue microenvironment could potentially open the door to novel therapeutic targets intervention in hepatitis and other disease states. **Financial support:** CAPES (Brazil), CIHR/IRSC and Alberta Innovates Health Solutions (Canada). All experiments involving animals were approved by the University of Calgary Animal Care Committee (protocol numbers MO8131 and MO7098) and were in compliance with guidelines established by the Canadian Council for Animal Care.

04.036 Liposomes of phosphatidylserine inhibit the respiratory burst induced by LPS, Zymosan or PMA in murine macrophages. Charão CCT[1], Assreuy J[2] [1]UFSC – Ciências Fisiológicas, [2]UFSC – Farmacologia

Introduction: Phosphatidylserine (PS) is expressed on the outer leaflet of plasma membranes of apoptotic cells serving as an “eat me” signal to macrophages. Macrophages in phagocytic activity generate a respiratory burst by increasing oxygen consumption and the production of superoxide anion. A similar burst can also be induced by soluble stimulus such as phorbol 12-myristate 13-acetate (PMA) or bacterial endotoxin (LPS). In the present work we studied the effect of PS liposomes in the respiratory burst caused by zymosan, LPS+IFN and PMA in murine (C57/Bl6) peritoneal macrophages elicited by thioglycollate. As a control, liposomes of phosphatidylcholine were used. **Methods**: Murine peritoneal macrophages ($4x10^6$/mL) were incubated with PS or PC liposomes (370 µg/mL) for 24 h, washed with Hank's and activated with LPS (100 ng/mL) plus IFN or zymosan (5 particles/cell) or PMA (2.4 mg/mL). Respiratory burst was measured by luminol chemiluminescence (50 µM).

Results: Peritoneal macrophages exhibited increases in luminol chemiluminescence reaching a peak 30 minutes after activation and decaying back to basal levels within 60 minutes. When activated by LPS+IFN the counts raised from 250 to 2828 cpm; zymosan from 11700 to 126600 cpm and PMA from 410 to 303000, 30 min after the addition of the activator. Incubation with PS for 24 hours resulted in a significant inhibition of respiratory burst (LPS+IFN was reduced to 190 cpm; zymosan to 200 cpm and PMA to 20000 cpm). PC liposomes were without effect. **Conclusion**: Macrophage activation with particulate or soluble stimuli induced a significant respiratory burst. However, zymosan and PMA were much more effective to do so compared with LPS. When cells were incubated with PS liposomes 24 hs before, a huge inhibition of the burst was observed for all stimuli. Since PC liposomes were ineffective in this regard, our results suggest that apoptotic cells (or PS liposomes) exert a potent inhibitory effect on the macrophage respiratory activation. It would be important to study the mechanism by which PS liposomes display this inhibitory activity. **Financial Support**: CNPq, CAPES, FINEP and FAPESC. Research approved by the Institutional Animal Ethical Comittee: CEUA/UFSC PP790

04.037 Mast cells involvement in the inflammatory process and local renin angiotensin system components expression on gingival tissue of diabetic mice with periodontal disease. Queiroz DPS[1,2], Brito VGB[3,2], Pereira JP[3], Beltran CT[3,2], Vieira LV[3,2], Lara VS[4], Santos CF[5], Oliveira SHP[3] [1]Unesp-Araçatuba – Ciências Básicas, [2]Multicenter Graduate Program in Physiological Sciences, [3]FOA-Unesp – Ciências Básicas, [4]FOB-USP – Estomatologia, [5]FOB-USP – Ciências Biológicas

Introduction: Previous studies showed a potential participation of local Renin Angiotensin System (RAS) on inflammatory process of periodontal diseases (PD) (Santos *et al*. PLosOne, 10 (8):e0134601, 2015). So, the present study aimed to evaluate the role of mast cells upon inflammatory process, bone resorption, tissue repair and local RAS components production in gingival tissue (GT) of normal (NM) and diabetic mice (DM), depleted or not of mast cell (MC) submitted to experimental periodontal diseases (PD). **Methods:** Male Balb/c mice were used. Diabetes was induced by streptozotocin (200 mg/Kg, IP) and MC depletion was conducted by pre-treatment with compound 48/80. NM and DM, MC depleted or not, were subjected to 15 days of PD (bilateral silk wire ligature placed on the first inferior molars). Hemimandibles were harvested for histomorphometry and attached gingiva was used for gene expression analysis of angiotensinogen (*Agt*), angiotensin I-converting enzyme (*Ace*), angiotensin II Receptor type 1 and 2 (*Agtr1*, *Agtr2*), fibronectin (*FN*), collagen (*Col1a1, 2a1,* and *3a1*) and *Tgfβ1* by real-time RT-PCR. Animal Research Ethical Committee Approved Process #00106-2016. **Results:** Histomorphometry showed an intense neutrophil recruitment on the furcation region of alveolar bone on NM and DM with PD, and 48/80 pre-treatment leaded to reduce neutrophil infiltrate in both group. Alveolar bone resorption on PD groups was observed to be slightly increased in DM compared to NM, and mast cell depleted groups did not showed differences regarding this parameter. Regarding RAS components expression, PD was able to increase *Agt* expression on NM and DM, and this expression was more accentuated in 48/80 treated groups. Regarding *Ace* expression, we observed an increase in the PD group, and MC depleted DM had a significant decrease of *Ace* expression. *Agtr1* expression was increased only in DM with PD, however the absence of MC decreased this response. The *Agtr2* expression was increased in NM with PD, and the absence of MC decreased its expression. *Agtr2* expression was constitutive in DM and PD or MC depletion did not cause significant alterations. There was an increase on FN, Col1a1, Col1a2 and Col1a3 expression in NM with PD and it was potentiated in DM with PD. The absence of MC decreases the level of tissue repair markers expression in DM, but not in NM. The *Tgfβ1* expression decreased with PD, and the absence of MC did not alter this response. **Conclusion:** In summary, our preliminary data suggest that PD enhances alveolar bone resorption and neutrophil accumulation on furcation area, decrease tissue repair marker and increase RAS-derived inflammatory components in GT of NM and DM. Furthermore, MC potentiates the bone resorption and inflammatory infiltrate in NM and DM. They also decrease the tissue repair and RAS-derived inflammatory components only in DM. In conclusion, the presence of MC can alter the inflammatory response and tissue repair probably induced by RAS from GT mainly in DM. **Acknowledgment:** FAPESP (Grant #2015/03965-2) and CAPES, for Financial Support.

04.038 Cross-talk between protease activated receptor (PAR)2 and toll-like receptor (TLR)4 on phagocytosis of zymosan and inflammatory activity of murine peritoneal macrophages. Barra A, 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 leukocytes. 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,1µg/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 after 24 hours of stimulation with LPS, followed by incubation with SLI (30µM,1h) or TRYP (10nM,1h). Statistical analyses were performed using one-way ANOVA followed by Tukey post-test. **Results:** SLI or TRYP incubation increases PI in macrophage from wild mice (DMEN, 0.4500* ± 0.04041; DMEN+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 (DMEN, 1.407* ± 0.1345; DMEN+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 DMEM+LPS. SLI reduced nitrite production in LPS-stimulated macrophage (µM) at 24h of the culture in macrophage from wild mice (DMEN, 36.39 ± 4.709; SLI, 28.78 ± 2.798; LPS, 32.31 ± 8.211; LPS + SLI, 6.420* ± 1.483) and TLR4 KO mice (DMEN, 36.39 ± 4.709; SLI, 28.78 ± 2.798; LPS, 32.31 ± 8.211; LPS + SLI, 6.420* ± 1.483) * $p<0.05$ when compared to LPS+SLI. **Discussion:** In vitro PAR2 activation was able to increasing phagocytosis in LPS-stimulated peritoneal macrophages, in addition, was able to reduce the NO production. Taken together our results suggest a role for PAR2 and TLR4 modulating two important mechanisms of macrophage repertory. **Conclusion:** Our data demonstrate a role for PAR2 and TLR4 on the macrophage activation, suggesting these receptors as a potential targets to the treatment of inflammatory diseases. **Financial support:** CNPq, Fapemig. Experimental procedures were approved by the local animal ethics committee (certificate number 374/2014)

04.039 Beneficial effects of a H_2S-releasing dexamethasone derivative on atopic dermatitis in mice, Coavoy-Sánchez SA[1], Cerqueira ARA[1], Teixeira SA[1], Soares AG[1], Santagada V[2], Caliendo G[2], Costa SKP[1], Muscará MN[1] [1]ICB-USP – Pharmacology, [2]University of Naples – Pharmacy

Introduction: Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by pruritic and eczematous skin lesions, associated with enhanced T-helper2 (Th2) lymphocyte response that results in elevated serum Immunoglobulin E (IgE) levels[1]. It affects 30% of children but is also highly prevalent in adults[2]. Previous studies from our laboratory showed that hydrogen sulfide (H_2S) donors reduce skin inflammation and both histaminergic and non-histaminergic pruritus in mice[3,4]. Since the therapeutical potential of H_2S donors on AD has not yet been studied, we decided to investigate the comparative effects of dexamethasone and a H_2S-releasing derivative on the development and clinical signs of AD induced by 2,4-dinitrochlorobenzene (DNCB) in mice. **Methods:** Female BALB/c mice (6-8 week-old) had their dorsal skin regions shaved and 200 ul of 0.5% DNCB in acetone/olive oil (3:1) were topically applied during 3 consecutive days. On days 15, 17, 19 and 22, the mice were challenged with 200 ul of topical 0.2% DNCB on the dorsal skin and 20 ul of 0.2% DNCB on the right ear. On days 19-23 after sensitization, mice were topically treated with equimolar doses of dexamethasone (0.1% and 0.05%) or the H_2S-releasing derivative (0.14% and 0.07%). Skin severity score and scratching behavior were assessed before each challenge and before euthanasia on day 24th. Blood samples were collected for cell counting and serum IgE analysis. The spleens were also collected for recording of their weights and the total number of splenocytes. **Results:** The continuous application of DNCB resulted in AD-like skin lesions, scratching behavior, elevated serum IgE, augmented spleen weight and increased total number of splenocytes, and treatment with 0.14% or 0.07% H_2S-dexamethasone significantly reduced the skin severity score (28.5% and 27.5% respectively; $P<0.001$), scratching behavior (50.6% and 88.8% respectively; $P<0.001$), spleen weight (313.4% and 266.2% respectively; $P<0.001$) and total number of splenocytes (386.6% and 377.5% respectively; $P<0.001$). These effects were not significantly different to those observed in the dexamethasone-treated group, except for skin severity score, for which 0.14% H_2S-dexamethasone was significantly more effective than the parent drug (28.5% vs. 10.2% reduction; $P<0.05$). Conclusions: The above shown results evidence the that topical application of H_2S-releasing dexamethasone has beneficial effects on the clinical sings of AD, and based on this, we can suggest that this compound may represent a potential therapeutical alternative for treatment of AD. **Financial Support**: CAPES, CNPq, FAPESP. Animal Research Ethical Committee: CEUA - ICB/USP; n° 129/2016. References: [1]Ghazvini P et al. J Pharm Pract. 2010;23(2):110. [2]Bieber T. N Engl J Med. 2008;358(14):1483. [3]Rodrigues L et al. Pharmacol Res. 2017;115:255. [4]Coavoy-Sánchez SA et al. Pharmacol Res. 2016;113(Pt A):686.

04.040 Potential anti-inflammatory effect of LQFM-021 in carrageenan-induced inflammation: **The role of nitric oxide**. Florentino IF[1], Silva DPB[1], Silva DM[1], Cardoso CS[1], Moreira ALE[2], Borges LB[2], Soares CMA[2], Carvalho PMG[3], Lião LM[4], Ghedini PC[1], Menegatti R[5], Costa EA[1] [1]UFG – Farmacologia, [2]UFG – Bioquímica, [3]UFOB, [4]UFG – Química, [5]UFG

Introduction The pyrazole compound LQFM-021 exhibits vasorelaxant, antinociceptive and anti-inflammatory activities. Furthermore, it has low toxicity, indicating that this compound may be a good prototype for the development of new analgesic/anti-inflammatory drugs. Therefore, the aim of this study was to investigate the potential anti-inflammatory activity of LQFM-021 using a model of carrageenan-induced inflammation as well as the mechanism of action and role of nitric oxide in this effect. **Methods** Male Swiss albino mice weighing approximately 30 g were used in this study; the experimental protocol was approved by the Research Ethics Committee of UFG (number 017/13). Pharmacological methods: carrageenan-induced paw edema and pleurisy tests. **Results** Acute treatments with LQFM-021 (30 and 60 mg/kg p.o.) reduced paw edema formation from the 2nd hour in a dose-dependent manner. Over this time, reduced edema by 25.3% ($P \leq 0.01$) and 33.7% ($P \leq 0.001$), respectively. After the 3rd hour, the same doses reduced edema by 25.5% and 33.0% ($P \leq 0.01$), respectively. At the 4th hour, reduced edema by 30.4% and 29.0 % ($P \leq 0.05$), respectively. In the carrageenan-induced pleurisy test, LQFM-021 (30 mg/kg p.o.) reduced the leukocyte (polymorphonuclear) count in the pleural cavity by 38.2 % ($P \leq 0.01$), as well as decreased protein extravasation, by 30.9 % ($P \leq 0.01$), and myeloperoxidase activity, 43.0 % ($P \leq 0.05$). This dose of LQFM-021 increased the NO (nitrite/nitrate) levels by 102 % ($P \leq 0.01$) and IL-4 levels by 261.42 % ($P \leq 0.001$), in the pleural cavity. Besides decreased the TNF-α and IL -1β levels by 55.6 % ($P \leq 0.01$) and 26.38 % ($P \leq 0.05$), respectively. Moreover, pre-treatment with L-NAME reversed the effect of LQFM-021 on NO, leukocyte migration, and the TNF-α and IL-1β levels. Additionally, we observed that LQFM-021 showed weak inhibitory activity on cyclooxygenases, but reduced the PGE_2 levels by 57.7 % ($P \leq 0.001$), in the pleural cavity. Immunoblot analyses showed that LQFM-021 promoted a decrease in COX-2 levels and increase in iNOS levels. **Conclusion** In conclusion, we demonstrated that LQFM-021 has marked anti-inflammatory activity by reducing polymorphonuclear recruitment, which is associated with the inhibition of the production of inflammatory cytokines and eicosanoids. In addition, we found that the synthase/release of nitric oxide promoted by LQFM-021 is essential for the anti-inflammatory effect observed. **Sources of research support:** CNPq and CAPES. **Research Ethics Committee of UFG:** Number 017/13

04.041 Early mice exposure to the ambient pollutant 1,2-napththoquinone impairs adhesion molecules expression in the airways. Feitosa KB[1], Cunha AC[1], Santos KT[1], Favaro RR[2], Santana FPR[3], Prado CM[4], Zorn TMT[2], Muscará MN[1], Costa SKP[1] [1]ICB-USP – Farmacologia, [2]ICB-USP – Biologia celular e Desenvolvimento, [3]Unifesp-Diadema – Biociências, [4]Unifesp – Biociências

Introduction: We have previously shown that the early contact with the ambient pollutant 1,2-naphthoquinone (1.2-NQ) led to a marked enhancement of the innate immune responses, characterized by exacerbation of the pulmonary allergic inflammatory response and airway remodeling (mucus hyper secretion and increased airway smooth muscle thickness) at adulthood via a mechanism dependent on activation of endotoxin-toll-like receptor (TLR4) [1, 2]. This study was carried out to further characterize the fibrosis and to evaluate whether early exposure to 1,2-NQ impairs leukocyte-adhering ability on TLR4-KO mice by down-regulating adhesion molecules. **Methods**: Male C57BL/6 WT and TLR4 KO neonate mice were exposed to the pollutant 1,2-NQ (100 nM) or its vehicle, accordingly [1]. Mice were divided into control (vehicle), pollutant (1.2-NQ), allergic (OVA) and 1.2-NQ+OVA groups. At adult period, mice were killed and biological (lung) samples were collected and processed histologically in order to permit the analysis of pulmonary fibrosis (picrosirius red stain) and the expression of the adhesion molecules: Vascular cell adhesion molecule-1 (VCAM-1), Intercellular Adhesion Molecule 1 (ICAM-1) and *Platelet* endothelial cell adhesion molecule-1 (*PECAM-1*) via immunostaining technique. **Results**: The allergic insult with OVA in WT prior exposed mice to 1,2-NQ did not promote increased collagen fibers deposition in the lung vessels or airway compared to WT allergic control mice. In WT mice, the OVA challenge led to increased collagen deposition in lung vessels (31,4±0,97%) compared to control group (vehicle; 14,2±2,6%; n=4). The VCAM-1 expression in the lung parenchyma and peribronchiolar area (0,80±0,10 and 4,54±0,9%, respectively) of WT mice prior exposed to 1,2-NQ and OVA increased significantly compared to respective OVA or vehicle-treated group (0,24±0,03 and 0,33±0,1%, respectively; n=4) as well as when compared to allergic TLR4 KO mice prior exposed to 1,2-NQ. The allergic challenge with OVA in WT adult mice exposed to 1,2-NQ as neonate has also led to a marked immunoreaction for PECAM-1 in the parenchyma (1,1±0,1%; n=7) as compared to OVA, vehicle or 1,2-NQ-treated mice (0,2±0,1, 0,6±0,1 e 0,5±0,2%, respectively; n=4). The ICAM-1 immunoreaction in the lung of WT and TLR4 KO mice prior exposed to 1,2-NQ and OVA did not differ from each other. **Conclusion**: Our results imply that TLR4-signalling pathway potentially helps to suppress the exacerbation of allergic lung inflammation upon early exposure to 1,2-NQ without affect fibrosis remodeling via impairment of adhesion molecules (PECAM-1 and VCAM-1), known as important step in the endothelium adhesion and transmigration of leukocytes. Acknowledgments: CAPES, CNPq, FAPESP. Ethic committee: Number 48/2016 CEUA **References: 1.** Inflammation Research. 2011 v. 60. p. S174-P276. 2. 48th Brazilian Congress of Pharmacology and Experimental Therapeutics and 21st Latin American Congress of Pharmacology. 2016, 04.035, p. 61.

04.042 Involvement of Adenosine A2A receptor in the lung fibrosis caused by silica particles in mice. Silva PMR[1], Jannini-Sá YAP[1], Savio LEB[2], Coutinho-Silva R[2], Carregaro V[3], Alves-Filho JC[4], Martins MA[1] [1]Fiocruz – Inflamação, [2]UFRJ – Imunofisiologia, [3]FMRP-USP Bioquímica e Imunologia, [4]FMRP-USP – Farmacologia

Introduction: Adenosine is a nucleoside that has been reported to be implicated in fibrosis, being considered as a potential therapeutic target for fibrotic diseases. In this study, we investigated the involvement of adenosine in pulmonary fibrosis in silicotic mice. Lung fibroblast reactivity was also evaluated *in vitro*. **Methods:** Mice were instilled with silica and the analyses performed 7 and 28 days later. The parameters included lung function (resistance and elastance) and tissue morphology/morphometry. Lung fibroblast reactivity (proliferation and MCP-1) was evaluated *in vitro*. **Results:** Expression of CD39 and CD73 enzymes, responsible for adenosine generation, was increased in the lungs at 7 days of silicosis. Adenosine receptor expression was altered in the lung of the silicotic animals, reflecting increase of A_1 and A_{2B} receptors, reduction of A_{2A} receptor and no alteration of A_3. Lung fibroblasts stimulated with IL-13 and adenosine, alone or in combination, led to an increase in proliferation and MCP-1 production, phenomena sensitive to A_{2A} receptor antagonists ZM 241385 and SCH-58261 Fibroblasts from A_{2A} receptor knockout mice were less responsive to IL-13 and adenosine stimuli. CD39 and CD73 inhibitors also suppressed IL-13 stimulated fibroblast proliferation. Receptor A_{2A} silicotic knockout mice showed reduction of lung function decrease and fibrotic granulomatous responses. **Conclusion:** Our results show that adenosine seems to be implicated in the fibrotic response associated with silicosis in mice, by a mechanism, at least partially dependent on its ability to synergize with IL-13 and its action on A2A receptors. **Financial support:** CNPq, FAPERJ, CAPES.

04.043 N-acetylcysteine prevents the decreased carotid occlusion time in mice injected with lipopolysaccharide. Caloi CM[1], Vicente C[2], Pereira DS[3], Werneck CC[3], Naime ACA[1], Marcondes S[1] [1]Unicamp – Farmacologia, [2]Unicamp – Biologia Estrutural e Funcional, [3]Unicamp – Biologia Funcional e Molecular

Introduction: Endotoxemia is a systemic inflammatory reaction that is considered a classic condition of oxidative stress, either by increasing reactive oxygen species (ROS) production, or by reducing the expression and/or activity of antioxidant systems. Lipopolysaccharide (LPS) is widely used to study the endotoxemia, since its administration to animals leads to some symptoms observed in this condition such as increase of ROS levels and disseminated intravascular coagulation. N-acetylcysteine (NAC) is an antioxidant that, besides to be a direct ROS scavenger, is also a precursor of glutathione synthesis, a endogenous antioxidant. Therefore, the objective of the present work was to investigate the effect of NAC on the occlusion time, coagulation cascade and platelet aggregation of LPS-inject mice. **Methods:** The present study was approved by the Committee for Ethics in Animal Research (State University of Campinas – UNICAMP, protocol number 3764-1). Males C57 Black6J mice were injected with saline or LPS (1 mg/kg, i.p.) and at 2, 6, 24 and 48h thereafter arterial blood was collected. In some experiments, mice were treated with NAC (150 mg/kg, i.p.) 30 min after saline or LPS injection and then, 6h later the blood was collected. In the acclusion assays, mice were anesthetized with chloride of ketamine and xylazine and the thrombus formation in the carotid was induced by a filter paper saturated with ferric chloride and it was determinated using a ultrasound probe. Coagulation time was measured in a coagulometer. Platelet counts were carried in peripheral blood using Newbauer chamber. Washed platelet aggregation was evaluated in a two-channel aggregometer. The statistical significance between groups was determined by using ANOVA followed by the Tukey test. **Results:** In the group injected with saline, the vessel occluded in 390±22 seconds. The carotid occlusion in mice 2h, 24h e 48h after LPS injection was not different than that one injected with saline (occlusion time 389±16, 413±29 and 343±9 sec, respectively). However, the occlusion time was significantly reduced in mice 6h after LPS injection (273±1,4 sec), which was reversed by NAC (536±67 sec). Activated partial thromboplastin time (aPTT) was higher in LPS (6h) than in saline group, however prothrombin time (PT) was significantly lower in mice 6h after LPS injection (reduction of 48% compared to saline-injected mice). Either platelets counts or aggregation was significantly reduced in mice 6h after LPS injection compared to saline group (decrease of 36% and 65% in platelet number and aggregation, respectively). **Conclusions:** Reduced occlusion time in mice 6h after LPS injection is not depend on platelets number or aggregation, as well as on intrinsic pathway of blood clotting, but extrinsic pathway probably takes part in this effect. In addition, NAC prevents the reduced occlusion time observed in mice exposed to LPS. **Financial Support**: CNPq, FAPESP.

04.044 Biocompatibility evaluation of polypyrrole polymer using zebrafish as a model organism. Costa KM[1], Pereira TCB[2], Valente CA[3], Soares JC[2], Cruz FF[2], Basso NRS[4], Bogo MR[2] [1]PUCRS – Ciências da Saúde, [2]PUCRS – Biologia Celular e Molecular, [3]PUCRS, [4]PUCRS – Química

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

04.045 Siglec-5 Activation by Alpha-1-Acid Glycoprotein (AGP) inhibits neutrophil actin polymerization. Lorenzini CB[1], Cardoso F[1], Colon D[2], Cunha FQ[2], Spiller F[1] [1]UFSC – Farmacologia, [2]FMRP-USP – Farmacologia

Introduction: Neutrophil recruitment has a central role in host response and resolution of inflammation, however, if uncontrolled can lead to severe tissue damage. In response to a chemotactic gradient, neutrophils are activated inducing F-actin polymerization leading them to extravasate and chemotax toward to the site of inflammation. In this context, neutrophils must be tightly regulated to prevent unwanted damage caused by an exacerbate inflammatory responses. Siglec-5 and Siglec-9, members of the Siglec (sialic-acid-binding immunoglobulin-like lectin) family are inhibitory receptors expressed on neutrophils and when activated down regulate the inflammatory response. Therefore, we hypothesized that Siglec-5 and/or -9 activation inhibits the mechanisms related to human neutrophil chemotaxis. **Methods:** Human neutrophil chemotaxis response was induced by fMLP (10-7M) in a Boyden chamber for 1 h at 37°C. The actin polymerization was stimulated by fMLP (10-7M) for 3 minutes, and F-actin stained with Phalloidin Rhodamin for the immunofluorescence assay. To observe whether AGP sialic acid residues influence on the inhibitory effect, an AGP treated with neuraminidase (cleaves sialic acid in α-2,3 - α-2,6- or α-2,8) group was included in all the experiments. **Results:** AGP (500 µg/ml) inhibited human neutrophil chemotaxis and actin polymerization in vitro induced by fMLP chemotactic factor. In addition, AGP-treated neuraminidase (which cleaves sialic acid) significantly reversed this protein suppressive effect. Furthermore, neutrophils pre-treated with blocking antibody anti-Siglec-5, but not with anti-Siglec-9, prevented the AGP inhibitory effect on actin polymerization. Moreover, we characterized AGP as a ligand for Siglec-5. **Conclusion:** Our results showed that AGP sialic acid residues are involved in neutrophil chemotaxis and actin polymerization inhibition, and the inhibitory effect on actin polymerization occurred via Siglec-5 activation. **Financial Support**: CAPES, CNPq, FAPESP and PPGF-UFSC Research approved by the Human Research Ethical Committee under the number: UFSC, n°283/08

04.046 Hydrogen sulfide inhibits histopathologic changes in mouse model of asthma. Villela Filho GJM, Colombo FF, Araújo VC, Passador-Santos F, Renno AL, Ferreira HHA São Leopoldo Mandic Center

Introduction: The mechanisms underlying airway hyperresponsiveness remain unclear, although airway inflammation and remodeling are likely important contributing factors. Recent studies showed that endogenous hydrogen sulfide (H_2S) plays an anti-inflammatory role in acute allergic airway inflammation in mice. In this study, we utilized a mouse model of chronic OVA-allergen induced airway remodelling to determine whether exogenous H_2S could reduce airway remodelling. **Methods:** All experiments were approved by the Animal Ethics Comitee/SLMandnic (LICENSE N. 2015/0457). Balb/c mice were sensitized by subcutaneous (s.c.) injection of ovalbumin (OVA; 4mg/ml) conjugated to aluminium potassium sulfate on days 1 and 11, besides intranasal (i.n.) OVA on day 11. Mice were, then, subject to a chronic i.n. allergen exposure protocol that was comprised of a six 2-days period of OVA administration (100 µg in 25 µl saline), each separated by 12 days. Thirty min before each OVA-challenge, mice received the NaHS-treatment (140 µmol/kg/day, i.p.; H_2S donor). Left lungs were removed and immediately fixed in formalin for preservation of pulmonary architecture and posterior inclusion in paraffin. Five µm thick sections were stained with hematoxylin-eosin, Masson's trichrome, toluidine-blue and immunostained using monoclonal antibodies against α-smooth muscle actin. The peribronchial segments were analysed for the quantification of inflammatory infiltration including eosinophils. The extra cellular matrix of bronchiolar segments and bronchiolar smooth muscle were analyzed by measuring the average of ten diameters of positive areas. All analyzes were performed under a light microscope attached to an image analysis system (ImageJ). **Results:** Using airway morphometry we demonstrated that chronic OVA-challenged provoked significantly increased levels of lung leukocyte and eosinophilic infiltration besides features of airway remodelling including increased peribronchial extra cellular matrix and thickness of the peribronchial smooth muscle layer. In contrast, NaHS-treated mice significantly reduced peribronchial leukocyte and eosinophils infiltration. NaHS mice also had significantly reduced thickness of peribronchial smooth muscle layer and peribronchial extra cellular matrix. **Conclusion:** Our study provided novel evidence that H_2S can be protective in allergic asthma by reducing structural changes of remodelling and could be a therapeutic target for alleviating asthma symptoms. **Financial Support**: CNPQ All experiments were approved by the Animal Ethics Comitee/SLMandnic (LICENSE N. 2015/0457).

04.047 Regulatory T cells accumulate in the injured nerve and reduce neuropathic pain by suppression of TH1 cells. Davoli-Ferreira M, Lima KA, Fonseca MDM, Guimarães RM, Quadros AU, Cunha TM FMRP-USP

Introduction: Neuropathic pain is a debilitating condition caused by damage to the somatosensory nervous system, such as peripheral nerve injury. The immune system, and in particular the adaptive T cell response, plays a key role in mediating such pain. Regulatory T (Treg) cells are a small subpopulation of inhibitory T cells that prevent autoimmunity, limit immunopathology and maintain immune homeostasis. It was recently shown that Treg cells reduce neuropathic pain following peripheral nerve injury; however, the mechanisms by which these cells act remain unclear. **Methods and Results:** Here, we showed that adoptive transfer of $Foxp3^+$ cells or pharmacological expansion of this cell subpopulation reduces hyperalgesia post-PSNL (partial nerve sciatic ligation); meanwhile, $Foxp3^+$ cells depletion significantly increases neuropathic pain, followed by a massive leukocyte infiltration, in particular T $CD4^+$ lymphocytes, at the site of the injury. Although the transcriptional profile of anti-inflammatory cytokines was not changed in nerves of mice with no $Foxp3^+$ cells, we observed increased levels of IFNγ and T-bet transcripts, but not Rorgt, IL-17 and GATA-3, which suggests that Treg could modulate T_H1 response during nerve injury and consequently reduce hyperalgesia. In contrast to previous reports, we did not find Treg infiltration in dorsal root ganglia (DRG) and spinal cord post-PSNL, however Treg absence was responsible to increase the levels of IBA-1 and ATF3 at DRG, showing that peripheral inflammation observed Foxp3-deficient mice could induce activation of DRG resident cells. **Conclusion:** Altogether, our data show that regulatory T cells acts at the site of the injury reducing Th1 immune response and indirectly controlling DRG resident cells activation indirectly, which contributes to reduction of neuropathic pain development and maintenance. **Financial Support**: FAPESP, CAPES, CNPq Animal Research Ethical Committee: 161/2015 (Ribeirão Preto Medical School)

04.048 Antioxidant activity of *Aedes aegypti*'s saliva in murine model of sepsis.
Monterio VVS[1], Gomes RS[1], Navegantes KC[1], Reis JF[1], Oliveira ALB[1], Rodrigues DVS[1], Romao PRT[2], Monteiro MC[1] [1]UFPA – Farmácia, [2]UFCSPA – Biomedicina

Sepsis is characterized by a potentially fatal organ dysfunction caused by a dysregulated immune response to infection. Despite having a well-established treatment sepsis is still an important public health problem, presenting a high index of mortality and morbidity, making necessary the search for new therapies for its treatment. *Aedes aegypti* saliva (SV) has several components with immunomodulator and anti-hemostatic activities. This study aims to evaluate the effects of SV in animals with sepsis. To this study were used 48 Swiss male mice weighing between 20 and 30g. The animals were pretreated intraperitoneally with 0.9% saline, Ceftriaxone (CEF) (20 mg/kg) or *Ae. aegypti* salivary gland (3.5 µg/ml) 1 time per day for 2 days and submitted to Cecal ligation puncture procedure (CLP) or only received the surgical procedure without induction of CLP (Sham). The animals were euthanized after 12 and 24 hours after induction of CLP for levels of nitric oxide (NO), malondialdehyde (MDA) and total antioxidant capacity in different organs and tissues. Another group was euthanized after 16 days (16 animals) to evaluate survival and weight. The performed procedures were approved by the Committee on Ethics in the Use of Animals (CEUA / UFPA - n° 1912080716). In the survival test, the animals treated with saline were killed between the 5th and 6th day, while the animals of the CEF group died between the 6th and 7th day, with a great loss of weight in both groups. The animals of the SV group survived until the 16th day, with weight loss in the initial days, with later weight recovery, comparable to the sham group. When NO production was evaluated, the saline group showed a marked increase in NO levels in all analyzed organs. The animals in the SV group presented reduction of NO levels in the blood, whereas in the peritoneal lavage these levels were increased when compared to the saline group. In the organs, the NO levels remained unchanged in 12 hours, when considered 24 hours, there was an increase in the heart when compared to the Salina group. Saline-treated animals also showed a significant increase in MDA levels in all analyzed parameters. Animals treated with CEF and SV had a reduction of these levels in all analyzed organs, and those in the SV group presented a more marked decrease in MDA levels mainly in the first 12 hours. Considering the total antioxidant capacity, the animals of the saline group present a significant reduction in the antioxidant capacity at all times evaluated in the blood and in the peritoneal lavage, while in the organs this decrease was not observed in relation to the Sham group. The animals treated with CEF and SV were able to revert the inhibition of the antioxidant capacity in the serum and in the peritoneal lavage. Considering the organs, the SV animals showed no significant difference in antioxidant capacity in the first 12 hours, and it was increased in all organs when 24 hours were evaluated. Treatment with SV demonstrated a beneficial effect in the treatment of sepsis and was able to improve the prognosis of the animals, increasing the survival and weight of these animals. Treatment with SV was also able to decrease NO and MDA levels, as well as increase the antioxidant capacity of CLP animals.

04.049 Leukocyte recruitment in Zebrafish: **a new tool for the study of inflammation and discovery of new drugs**. Charlie-Silva I[1], Prata MNL[2], Brasil AF[3], Melo DC[4], Corrêa JD[2], Belo MAA[1], Belo MAA[1], Manrinque W, Gomes JMM[2], Ferraris F[5], Conceição K[6], Lopes-Ferreira M[7], Klein A[3], Peres AC[2] [1]Unesp-Jaboticabal, [2]ICB-UFMG, [3]ICB-UFMG – Farmacologia e Fisiologia, [4]UFMG, [5]Fiocruz, [6]Unifesp, [7]IBu

Objective: Based on the importance of establishing new experimental models and the advantages of using zebrafish for screening new drugs with potential anti-inflammatory effects, and considering that their genetic homology is similar to that of humans, the present investigation evaluated leukocyte recruitment in the coelomic cavity of such fish. The effects of carrageenan on cellular accumulation in the exudate present in the cavity of zebrafishwere investigated. **Material and methods:** Thirty Daniorerio (± 1 g) zebrafish were randomly divided into three aquariums with 2.5 L of water (n = 10) to establish three treatments: T1 - Naive non-treated; T2 - PBS-inected; T3 - carrageenan-injected (CG) 3.5% in the coelomic cavity. Samples of exudate and blood were collected 1, 2, 3, 4, 5, 6 and 7 hours post-induction (HPI). Total leukocyte count was performed using a Neubauer chamber and flow cytometer. LC-MS/MS analysis of plasma 4h HPI was also conducted.**Results and Discussion:** The animals injected with CG exhibited a reduction in circulating leukocyte counts. Exudate analysis revealed an increase in the total number of (13.5 x105) cells accumulated in fish injected with CG 3.5% when compared with the response observed in the negative control fish (T2) in the cavity. Maximum values were reached after 4 hours, and subsequently decreased, returning to the initial values after six HPI. Differential count showed monocytes (dominant), followed by lymphocytes and granulocytes. After 4 hours of induction, we found that CG injection in the celomatic cavity revealed exudate and inflammatory mononuclear infiltrates in several organs correlating with the blood leukopenia of animals injected with 3.5% carrageenan. Proteomic analysis of fish plasma revealed differential expression of proteins between PBS and CG inejcted fish. **Conclusion:** Experimental models of inflammation in zebrafishhave not been well established and remains in the standardization phase.The present study identified that the inflammatory response was similar to findings in mammals and is related to the excessive recruitment of defense cells to the focus of the inflammation. Although some there are some gaps in knowledge of the underlying mechanisms involved in zebrafish model of inflammation, a better understanding of this model may be useful to their establishment as a new tool regarding the study of inflammation and the discovery of new anti-inflammatory drugs. **Financial Support:** FAPESP e FAPEMIG.

04.050 Antiprectic effect of citral during LPS fever. Emílio-Silva MT, Mota CMD, Hiruma-Lima CA, Antunes-Rodrigues J, Cárnio EC, Branco LGS Unesp

Introduction: Citral is a mixture of two monoterpenoid isomers (neral and geranial) widely used as a health-promoting food additive safe for humans and animals (approved by the U.S.FDA). *In vitro* studies have reported that citral reduces inflammation but nothing is known about its putative effect on fever. **Methods:** Rats were orally pretreated with vehicle (Tween 80%, 10 mL/kg) or citral (100 mg/kg) 30 min before LPS administration (100 µg/kg, i.p.) or saline (1 ml/kg, i.p.) and body temperature (Tb) measured. 90 minutes after administrations, the rats were decapitated for blood and anteroventral preoptic region of the hypothalamus (AVPO) collection for measurements of cytokines and prostaglandin E_2. **Results:** Citral caused no change in control euthermic rats (treated with Tween vs. Citral in 90 min.: 37,13 ± 0.18 vs. 37,20 ± 0,21ºC, respectively) but blunted fever (tween + LPS vs. citral + LPS in 90 min.: 38,76 ± 0,15 vs. 38,2 ± 0,15 ºC, respectively); plasma cytokine IL-1β, TNFα, IL-6 (tween + LPS vs. citral + LPS: IL-6 87,90 ± 27,73 vs. 23,07 ± 11,42 pg/ml; TNF-α 795,64 ± 296,79 vs. 193,78 ± 90,35 pg/ml; IL-1β 74,56 ± 29,92 vs. 22,97 ± 10,42 pg/ml, respectively) and production of plasma and AVPO PGE_2 (tween + LPS vs. citral + LPS: 296,56 ± 50,22 vs. 128,67 ± 38,33 pg/ml; 0,49 ± 0,14 vs. 0,16 ± 0,08 pg/ml, respectively). **Conclusion:** These data are consistent with the notion that citral plays a potent antipyretic role, acting on the peripheral febrigenic signaling (plasma levels of IL-1β, IL-6, TNF-α and PGE_2) and eventually down-modulating hypothalamic PGE_2 production. Support: *FAPESP (2015/22249-6), CNPq. Local Ethical Committee for Animal Use (No. 2015.1.788.58.5)*

04.051 Generation of PKM2 knockout HaCaT cell line by CRISPR/Cas-9 mediated genome editing. Públio GA[1], Cecílio NT[1], Vieira GV[2], Veras PV[1], Cunha Fernando Q[1], Cunha TM[1], Sales KU[2], Alves-Filho JC[1] [1]FMRP-USP – Farmacologia, [2]FMRP-USP – Biologia Celular e Molecular

Introduction/Aim: Keratinocytes are the cells that play a central role in skin inflammatory responses, participating in the development and maintenance of certain diseases such as psoriasis. It has been show that under inflammatory stimuli cells undergo a process of metabolic reprogramming, expressing genes involved in glycolysis such as PKM2. Recent studies have shown that PKM2 can be activated by inflammatory stimuli and is important for cytokine secretion and cellular proliferation. Results of our laboratory show that PKM2 is highly expressed in the skin of patients with psoriasis and animals with this disease. However, no studies in the literature show the participation of PKM2 in keratinocyte activation. Thus, the aim of this work is to generate a cell line of keratinocytes (HaCaT) knockout for PKM2 enzyme, as a tool for elucidate of the participation of PKM2 in keratinocytes activation, development and maintenance of psoriasis. **Methods/Results:** In this context, we used a method to selectively knockout PKM2 expression from mammalian cells using CRISPR/CAS9 technology. The CRISPR plasmid was generated by using a single-guide RNA targeting exon 10 of the PKM gene, with is responsible for the PKM2 expression, and then transfected several times in the HaCat cell line. Immunoblotting analysis showed that this processes impaired the PKM2 protein expression in some cells, but, consistent with an on-target effect, the PKM1 expression was not altered. **Conclusions:** Our data suggests that this powerful gene-editing technology can be used to dissect and analyze PKM2 signaling networks. **Financial Support:** FAPESP, CAPES, CNPq **Ethical Committe Lincense:** Animals (167/2015) / Human (CAAE56869316.3.0000.5440)

04.052 Comparison of immune-pineal axis activation in lethal and non-lethal LPS-induced endotoxemie Mori LT, Takiguchi RS, Markus RP, Fernandes PA IB-USP – Fisiologia

Introduction: Nocturnal melatonin synthesized by the pineal gland decreases leukocytes migration from blood to tissues. During the assembly of inflammatory responses, immune-related signals block the synthesis of melatonin by the pineal gland favoring leukocytes migration (da Silveira Cruz-Machado S, J Pineal Res 49, 183, 2010; Fernandes PA, J Pineal Res, 41,344, 2006). However, dysregulated inflammatory responses can lead to death and very little is known about the activation of the immune-pineal axis on these contexts. **Methods:** We evaluated the time course (1h - 6h), in male Wistar rats, of blood citokynes, melatonin and corticosterone levels in systemic sepsis-like inflammation induced by lethal and non-lethal doses of LPS (0,5mg/kg and 15mg/kg, i.v.) by ELISA. We also evaluated in blood and peritoneal phagocytes (CD11b/c+ cells) and Th lymphocytes (CD3/CD4+ cells) the levels of the melatonin biosynthetic pathway enzymes arylalkylamine N-acetyltransferases (AANAT), PAANAT and N-acetylserotonin O-methyltransferase and the melatonin receptors MT1 and MT2 by flow cytometry. Data are presented as mean ± sem. **Results:** Considering IL10 and IL6, both doses of LPS increased the production after LPS, but the effect was significantly greater in animals injected with the lethal dose of LPS after 2h (IL10: 197.6 ± 54.23 pg/mL vs 463.40 ± 65.39 pg/mL; IL6: 14201.7 ± 2709.0 pg/mL vs 49362.0 ± 8081.5 pg/mL; n = 4) and 6h (IL10: 181.4 ± 15.32 pg/mL vs 865.50 ± 106.40 pg/mL; IL6: 6754.9 ± 3943.34 pg/mL vs 166747.0 ± 19804.5 pg/mL; n = 4). Only the lethal dose of LPS induced an increase on IFNγ plasma levels after 6h of injection in comparison to saline group ($p<0.05$). The production of corticosterone was also potentiated in animals injected with the lethal dose of LPS after 2h of treatment in comparison to the non-lethal dose (131531.7 ± 15616.4 ng/mL vs 201201.8 ± 25856.9 ng/mL, n = 4). Confirming previously results, 0.5 mg/kg of LPS induced a persistent inhibition of melatonin in comparison to saline injected animals ($p<0.05$, One-way ANOVA). Interestingly, when animals were injected with a lethal dose of LPS there was an increase of plasma melatonin after 1h of treatment in comparison to the nonlethal dose (138.9 ± 19.06 pg/mL vs 554,7 ± 54.9 pg/mL, n = 4). The temporal patterns of expression in leukocytes of the melatonergic enzymatic pathway and melatonin receptors presented several differences in nonlethal and lethal LPS protocols. Considering melatonin receptors in peritoneal phagocytes, for example, we observed in non-lethal LPS treated animals a decrease of MT1 after 2h and an increase after 6h of treatment ($p<0.001$, One-way ANOVA) and the opposite profile when MT2 receptors were analyzed ($p<0.05$, One-way ANOVA), suggesting opposite regulation of melatonin receptors in these cells. Those variations were not observed in animals injected with the lethal dose of LPS. Discussion: The data indicate that the timing of the immune-pineal axis activation is altered in lethal inflammatory processes. The understanding of the role of the melatonergic system during these processes may lead to new therapeutic strategies to the treatment of uncontrolled systemic inflammatory pathologies. **Financial Support** from the São Paulo Research Foundation (FAPESP: 2015/23348-8, 2013/13691-1 and 2016/26081-5). CEUA-IBUSP Protocol: 207/2014.

04.053 Suppression by gold nanoparticles of silica-induced lung fibrosis in mice. Ribeiro NBS[1], Ciambarella BT, Arantes ACS, Serra MF, Azevedo R B, Fernandes AJM, Martins MA, Silva PMR Fiocruz – Farmacologia e Inflamação

Introduction: Inhalation of crystalline silica particles leads to development of silicosis, an occupational disease, is characterized by leukocyte infiltration, collagen deposition and granuloma formation. There is no efficient treatment available for fibrotic diseases, which demands the search for effective therapies. Remarkably, administration of gold nanoparticles (AuNPs) has been shown to present anti-inflammatory effects in different models of disease. Aim: In this study, we investigated the effect of aerosolized AuNPs on lung granulomatous fibrosis triggered by silica particles in Swiss-Webster mice. **Methods:** Anesthetized male Swiss-Webster mice received a unique intranasal (i.n.) instillation of silica particles (10 mg/50 µL) or vehicle (saline). AuNPs (0.3 - 60.0 µg/Kg) were aerosolized on alternate days during 7 days, starting 21 days post-silica instillation, and analyses were performed 1 day after the last dose of AuNP administration (28 days), including the following parameters: i) lung function (resistance and elastance) and airways hyper-reactivity to methacholine (3 - 81 mg/mL) by invasive plethysmography (Finepointe, Buxco System); ii) morphological alterations by classical histological techniques including staining with hematoxylin & eosin; iii) quantification of cytokine generation by ELISA. In parallel, evaluation of activation and survival of silica-stimulated alveolar macrophages (AMJ2C11 line) was also performed in vitro through quantification of TNF-α and Brdu test, respectively. All experimental procedures were approved by the Animal Ethics Committee of the Oswaldo Cruz Foundation (CEUA L-57/14) **Results:** We noted that therapeutic treatment of silicotic mice with AuNP led to partial inhibition of airway hyper-reactivity, which paralleled to a decrease in granulomatous response. Cytokine generation, mainly TNF-α, was reduced by AuNP. Intranasal instillation of TNF-α into normal mice led to airway hyper-reactivity, indicating that it can be an important therapeutic target in silicosis. In another set of experiments, we noted that alveolar macrophages treated with AuNPs showed reduced levels of TNF-α release and higher survival rate after silica challenge in vitro. Conclusions: Our results show that local treatment with AuNPs suppresses airways hyper-reactivity and lung fibrogenesis in silicotic mice, in a mechanism associated with reduction of tissue TNF-α generation. In addition, alveolar macrophage activation was also down-regulated by AuNPs, indicating that the cells can be considered as important target for the AuNPs. Additional experiments are needed in order to clarify better the effect of AuNPs in the context of silicosis. **Financial Support**: FIOCRUZ, CNPq, FAPERJ and CAPES. Key words: Lung, inflammation, fibrosis, therapy, gold nanoparticles.

04.054 *In vitro* evaluation of the anti-inflammatory activity of essential oil of *Cyperus articulatus* L. in macrophages. Ferreira JCC, Silva EBS, Almeida Junior JS, Barata LES, Moraes TMP, Pires-Moraes W UFOPA- Laboratório de Farmacologia Experimental

Introduction: The use of natural products, especially those derived from plants, is a traditional way of promoting disease relief, which has been used for more than five millennia in various civilizations. *Cyperus articulatus* L. belongs to the Cyperacea family, known in the state of Pará as Priprioca, which is popularly used in the treatment of headache, diarrhea, epilepsy, and parasitic diseases among others. **Objective:** Objective of this project was to evaluate the possible anti-inflammatory activity of the essential oil of *Cyperus articulatus* L (OECA) in cultures of macrophages stimulated with LPS and IFN-γ, through the cellular viability analysis and the dosage of inflammatory mediators (Nitric Oxide, TNF -α, IL-1B and PGE2) in order to contribute to the knowledge of the species and possible use as a pharmacological instrument for the treatment of inflammatory diseases by means of more effective drugs and with fewer adverse effects. **Methods:** The concentrations tested of OECA were 250, 500 and 1000 µg/mL. Cellular viability was performed according to the MTT method, the determination of nitric oxide production (ON) was performed by the Griess method. The leavel of cytokine interleukin 1β (IL-1β), tumor necrosis factor-α (TNF-α), Prostaglandins type 2 (PGE-2) were performed by ELISA method (CEUA Approval Number: 07004/2013). Analysis of the chemical composition of the oil was performed in the Agilent HP-6890 gas chromatograph equipped with an Agilent mass sorting detector, model HP-5975 using an HP-5MS capillary column. **Results:** The presence of monoterpenes, sesquiterpenes and sequiterpene ketones were observed as major constituents. We did not observe cell death of the groups treated with OECA. The essential oil of *Cyperus articulatus* L. (OECA) significantly reduced the production of Nitric Oxide, TNF-α, IL-1-β and PGE2, compared with groups stimulated with interferon-γ and Lipopolysaccharide (INF-γ + LPS) And treated with OECA. **Conclusion:** The results indicate that the OECA treatment exerts a potent anti-inflammatory activity, promoting the inhibition of inflammatory mediators. We also found that OECA did not demonstrate cytotoxic activity at concentrations lower than 2000 µg/mL. This work was funded by the Amparo Research Foundation of Para State.

04.055 Effect of *Tityus bahiensis* e *Tityus serrulatus* crude venom on platelet aggregation of rats. Morau MV, Naime ACA, Bueno PI, Bonfitto PHL, Marcondes S FCM-Unicamp – Farmacologia

Introduction: Scorpion accident is considered a serious public health problem in Brazil. *Tityus bahiensis and T. serrulatus* are the scorpion species responsible for the majority of scorpion sting accidents in Brazil and they are frequently found in São Paulo state. Symptoms of envenomation by *Tityus serrulatus* and *T. bahiensis* range from local pain to severe systemic reactions such as cardiac dysfunction and pulmonary edema. In addition, *Tityus serrulatus* induces hypercoagulability in rats at 15 and 60 min after intravenous administration, however there are no reports about the effects on platelets. Therefore, the aim of the present work was to study the effects of the *Tityus bahiensis* and *T. serrulatus* crude venom on platelet aggregation of rats. **Methods:** The lyophilized venom of *Tityus serrulatus* and *T. bahiensis* were provided by Instituto Butantan, SP, Brazil. The present study was approved by the Committee for Ethics in Animal Research (State University of Campinas – UNICAMP, protocol number 4314-1). Arterial blood was collected in ACD-C (9:1 v/v) from abdominal aorta of male Wistar rats (250-320 g). Platelet-rich plasma (PRP) was obtained after centrifugation of whole blood at 200g for 20 min and the platelets were washed using citrated buffer (pH 6.0). Washed platelets were suspended in Krebs's solution and the number adjusted to $2x10^8$ platelets/ml. Platelet aggregation was evaluated using a two-channel aggregometer **(**Chrono-Log Lumi Agregometer model 560-Ca, Havertown, PA, EUA). Platelets were incubated with crescent concentrations of the venom of *Tityus serrulatus* and *T. bahiensis* (1µg/ml - 300µg/ml) for different times (5, 15 or 30 min) before ADP addition. **Results:** Addition of *T. serrulatus* or *T. bahiensis* venom on washed platelets did not induce aggregation even at concentration as high as 300 µg/ml. *T. bahiensis* venom (300 µg/ml) incubated with platelets for 5 min did not affect ADP (5 µM)-induced aggregation. However, ADP (1 µM)-induced aggregation was significantly ($p<0.001$) reduced (reduction of 42%) by the incubation with *T. bahiensis* venom 300 µg/ml for 5 min, that was not increased at longer incubation time (15 or 30 min). On the other hand, incubation of *T. serrulatus* venom for 5 min dose-dependently inhibited ADP (1 µM)-induced platelet aggregation (inhibition of 10, 28 and 85% using 1, 10 and 100 µg/ml of venom, respectively). Aggregation induced by ADP 1 µM was abolished by *T. serrulatus* venom (300 µg/ml) incubated with platelets by 30 min. **Conclusion:** Venom from *Tityus serrulatus* inhibits more efficiently ADP-induced platelet aggregation than that one from *T. bahiensis*. *Tityus serrulatus* venom inhibits ADP-induced platelet aggregation in a dose- and time-dependent manner. **Financial support:** CNPq. **References:** Chippaux, J.P. Acta Trop., 107, 71, 2008. Lisboa, A. T. Toxicon, 94, 45, 2015. Lorenço W. R. Tropical Diseases, 21, 01, 2015. Severino, D. N. Inflammation, 32, 57, 2009.

04.056 Evaluation of anti-inflammatory and antioxidant activities of ethyl p-coumarate in acute inflammatory models. Gonçalves RLG[1], Lima-Filho ACM[1], Silva BG[1], Rezende DC[1], Silva IS[2], Sousa FBM[2], Sousa LKM[2], Medeiros JVR[2], Sousa DP[3], Oliveira FA[1] [1]UFPI – Research Center on Medicinal Plants, [2]UFPI – Experimental Physiopharmacology, [3]UFPB – Pharmaceutical Sciences

Introduction: The inflammatory process is the result of protection against antigens and the recovery of cellular damages. When uncontrolled, it can give rise to several diseases. Research into the anti-inflammatory and antioxidant effects of herbal products as adjuncts to the treatment of inflammatory disease is relevant because the non-steroids anti-inflammatory drugs (NSAID) and glucocorticoids have several adverse effects, such as gastric ulcers and metabolic disorders. Ethyl *p*-coumarate (*p*-CE*)* is a phenylpropanoid that has its antimicrobial activity described after isolation from *Tabebuia aurea* (Manso) S. Moore, but its anti-inflammatory action never has been evaluated. **Aims:** Therefore, this work aimed to investigate the effect of *p*-CE on inflammatory and oxidative stress parameters. **Methods:** Initially, the anti-inflammatory effect of *p*-CE was evaluated in Swiss mice (25-35 g), male and female, in carrageenan-induced paw edema model (1%, 50 µL).The animals were divided in vehicle, indomethacin (10 mg/kg, p.o.) and *p*-CE (50, 100, 150 and 200 mg/kg, p.o.) groups. In addition, peritonitis was induced in mice with carrageenan (500 µg/cavity) for counting of neutrophil and total leukocytes and evaluation of the activity of myeloperoxidase (MPO), glutathione (GSH) and malondialdehyde (MDA), in the groups according to pretreatment with vehicle with and without carrageenan (i.p.), indomethacin (10 mg/kg, p.o.) and *p*-CE (150 mg/kg, p.o.). **Results:** As results, it was observed that ethyl *p*-coumarate had significant inhibition of carrageenan-induced paw edema in all 4 hours of evaluation at doses of 150 and 200 mg/kg, compared to vehicle group. The *p*-CE (150 mg/kg) also significantly reduced total count of leukocyte and neutrophils (63.07 and 84.82% of ihnibition, $p<0.001$), MPO (0.487 ± 0.201 U/mL, $p<0.01$), MDA (1.452 ± 0.163 nmol/mL, $p<0.5$), and increase GSH (167.70 ± 26.08 µg/mL, $p<0.5$), compared to animals that received vehicle with carrageenan (2.585 ± 0.733 U/mL for MPO, 2.226 ± 0.133 nmol/mL for MDA and 86.76 ± 7.50 µg/mL for GSH), and similar to indomethacin group (0.575 ± 0.222 U/mL for MPO, 1.512 ± 0,240 nmol/mL MDA and 169.20 ± 25.53 µg/mL for GSH).**Conclusion:** Taken together, these results suggest that ethyl *p*-coumarate exhibits anti-inflammatory and antioxidant activity. **Financial support:** FAPEPI and CNPq. The study was started after obtaining approval from Institutional Animal Ethics Committee, of the Federal University of Piaui, approval Letter No. 138/16) **Keywords:** Phenylpropanoid. Ethyl *p*-coumarate. Anti-inflammatory activity. Antioxidant.

04.057 Regulation of Th17 cell differentiation by pyruvate kinase M2 supports autoimmune-mediated neuroinflammation. Damasceno LEA, Prado DS, Fonseca MDM, Veras FP, Cunha FQ, Alves-Filho JCF FMRP-USP

Introduction: Multiple Sclerosis is an inflammatory autoimmune disorder characterised by autoreactive Th17 cell response. Recent evidences demonstrate that Th17 cells undergo metabolic reprogramming, which is critical for their differentiation and effector function. The glycolytic enzyme Pyruvate Kinase-M2 (PKM2) converts phosphoenolpyruvate into pyruvate. Apart from its catalytic activity, it has been reported that PKM2 can be phosphorylated and translocated into the nucleus, controlling gene expression. Thus, this study aims to investigate PKM2 involvement in Th17 differentiation and its influence on the pathogenesis of Experimental Autoimmune Encephalomyelitis (EAE). **Methodology**: EAE was induced by immunising mice with MOG_{35-55}. Naïve T-$CD4^{+}CD25^{-}$ cells were cultured under Th17-polarizing conditions (IL-6–20ng/mL; TGF-b–2,5ng/mL). **Results:** By performing qPCR, immunofluorescence or immunoblotting, we found that PKM2 is overexpressed in dLNs and spinal cord of EAE-bearing mice, in which PKM2 phosphorylation degree was associated with disease severity. Treatment with shikonin (PKM2 inhibitor; $4mg.kg^{-1}$, s.c.) or CD4–specific *Pkm2* deletion ($CD4^{cre}PKM2^{flox/flox}$) noticeably reduced EAE symptoms along with a decrease of IL-$17^{+}CD4^{+}$ cells frequency and downregulation of inflammation-related genes in the spinal cord. *In vitro*, besides gene expression of PKM2 being augmented over differentiation, phospho-PKM2 was also highly expressed in Th17 lymphocytes. Moreover, inhibition or specific-deficiency of PKM2 reduced Th17 polarization. Interestingly, the absence of PKM2 in Th17 cells caused a decrease in expression of *Rorc* and *Rora* genes, which transcribes master transcription factors of Th17 cells. **Conclusion:** These findings imply an important role for PKM2 in autoimmune disorders by regulating Th17 differentiation. **Financial Support:** FAPESP **Animal Research Ethical Committee:** CEUA-FMRP 098/201

04.058 Establishment of an experimental model of emphysema: **Effect of the phosphodiesterase** (**PDE**) **4 inhibitor cilomilast**. Cunha LCL[1], Souza ET[2], Martins MA[1], Silva PMR[1] [1]Fiocruz, [2] Unime

Introduction: Chronic obstructive pulmonary disease (COPD) is a degenerative and irreversible dysfunction that has no effective treatment until now. PDE4 enzyme has been shown to be an important target in chronic inflammatory processes. Thus, this project aimed to establish an experimental model of emphysema in mice in order to further identify new PDE4 inhibitor compounds. **Methods:** Balb/c and C57Bl6 mice were intranasally instilled with elastase (PPE) (0.2 and 0.6 IU) and the following parameters were evaluated: i) pulmonary function (resistance and elastance) and airway hyper-reactivity to the bronchoconstrictor agent methacholine (invasive plethysmography); ii) morphology and morphometry (area of hyperinflation, elastic and collagen fibers); iii) quantification of tissue myeloperoxidase (MPO). All experimental procedures were approved by the Animal Ethics Committee of the Oswaldo Cruz Foundation (CEUA LW57/14). **Results:** We noted that the lung area of hyperinflation was shown to be of similar intensity when comparing both strains of mice used and to be dependent on the dose of PPE used. In parallel, we observed a significant increase in airways resistance and a decrease in the lung elastance. Treatment with the standard PDE4 inhibitor cilomilast reduced the area of hyperinflation, tissue levels of MPO and fibrosis, as well as improved lung function of C57Bl6 mice stimulated with PPE (0.2 IU). **Conclusion:** Our findings show that intranasal instillation of PPE in mice leads to a short-term and reproducible model of emphysema, which seems to be a useful tool when searching for anti-emphysematous compounds such as new PDE4 inhibitors. **Financial Support:** FIOCRUZ, CNPq, FAPERJ e CAPES (Brazil). Research Approval by the Animal Research Ethical Committee (process number): CEUA LW57/14.