04. Inflammation and Immunopharmacology

04.001 Identification of novel sulfonamide and sulfonilhidrazone derivatives active to accelerate resolution of silicosis in mice. Souza ET[1], Nunes IKC[2], Ferreira TPT[1], Ciambarella BT[1], Carvalho VF[1], Azevedo RB[1], Lima LM[2], Barreiro EJ[2], Martin MA[1], Silva PMR[1] [1]IOC-Fiocruz, [2]LASSBio-UFRJ – Avaliação e Síntese de Substâncias Bioativas

Introduction: Silicosis is an occupational disease characterized by an inflammatory component initiated by the deposition of silica particles in the lungs, followed by fibrosis and granuloma formation. There is no available treatment for the disease nowadays. PDE4 enzyme is considered an important therapeutic target in the case of chronic inflammatory dysfunctions. In this study, we evaluated the potential anti-inflammatory and anti-fibrotic effect of a new series of sulfonamide and sulfonilhidrazone compounds, planned to be PDE4 inhibitors, on the experimental model of silicosis in mice. **Methods**: A series of 13 derivatives was first screened for anti-PDE 4 activity by IMAPTM TR-FRET system, followed by testing their ability to inhibit LPS-stimulated alveolar macrophages (AMJ2C11 cell lineage) *in vitro*. For the silicosis model, Swiss-Webster mice received an intranasal instillation of silica particles (0.5 – 10 µm) or saline (controls). Therapeutic treatment consisted of an oral administration of the selected compounds (1 - 10 µmol/kg), every day from days 21 - 27 after silica challenge. The parameters analyzed included: i) lung function by invasive plethysmography (Finepointe, Buxco System); ii) lung tissue morphology and morphometry (H&E), cytokine generation (ELISA) and neutrophil infiltration (myeloperoxidase assay). Central adverse effects of nausea and vomiting, associated with PDE4 inhibition, were assessed indirectly by sleep induction caused by ketamine/xylazine. All experimental procedures were approved by the Committee on Use of Laboratory Animals of Oswaldo Cruz Foundation (license LW 57/14). **Results**: The anti-PDE4 screening of 13 sulfonamide derivatives revealed that LASSBio-1612, LASSBio-1628, LASSBio-1631 and LASSBio-1632 inhibited (≥ 40%) PDE4A1A and PDE4D3 activity, while no effect was noted on PDE4B1 and PDE4C enzymes. Furthermore, the four selected compounds were shown to suppress alveolar macrophage activation triggered by LPS as attested by significant decrease of TNF secretion. The therapeutic administration of LASSBio-1612, LASSBio-1628, LASSBio-1631 and LASSBio-1632 to silica-challenged mice led to suppression of lung function alteration (increased resistance and elastance) as well as airways hyper-reactivity to methacholine. The compounds also inhibited tissue neutrophil infiltration, increased cytokine production (TNF-α, INF-γ, KC, MIP-1 and MCP-1), fibrosis and granuloma formation. It is noteworthy that oral administration of the compounds had no central effect as compared to the classical inhibitor rolipram. **Conclusion**: Our findings demonstrate the effectiveness of four novel sulfonamide and sulfonilhidrazone derivatives (LASSBio-1612, LASSBio-1628, LASSBio-1631 and LASSBio-1632) in accelerating resolution of silica-induced inflammation and fibrosis in silicotic mice. The evidence present in this study also indicates that therapies aimed at inhibiting PDE4 could benefit patients with fibrotic diseases such as silicosis. **Financial support**: FIOCRUZ, FAPERJ, CNPq and INCT-INOFAR.

04.002 Quercetin therapeutically attenuates silica-induced pulmonary fibrosis in mice. Guimarães FV, Ferreira TPT, Ciambarella BT, Arantes ACS, Azevedo RB, Martins MA, Silva PMR Fiocruz

Introduction: Silicosis is a dysfunction caused by long-term inhalation of silica particles, characterized by intense inflammation and fibrosis, including the presence of granulomas in the parenchyma. In spite of the therapeutic arsenal currently available, there is no specific treatment for this disease. Quercetin is a flavonoid present in several plants including fruits, vegetables and some grains, which was shown to have important antioxidant and anti-inflammatory properties. **Aims**: This study was undertaken to investigate the effect of quercetin on experimental silicosis in mice. **Methods**: Swiss-Webster mice were instilled intranasally with silica particles (0.5 - 10 um) or saline (controls). Treatment with quercetin (2.5 - 10 mg/kg, p.o.) consisted of administration for 7 consecutive days, starting on day 21 post-silica. The classical antioxidant N-acetylcysteine (150 mg/kg) was used for comparison. All analyses were carried out 24 h after the last administration and oxidative, inflammatory and fibrotic markers were measured. All experimental procedures were performed in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (LW57/14). **Results**: We found that treatment with quercetin (5 and 10 mg/kg) attenuated leukocyte infiltration, tissue fibrosis (collagen deposition) and granuloma formation in the lungs of silicotic mice. The increased levels of cytokines (IL-1beta, TNF-alpha, KC, MIP1-alpha and MCP-1) in silicotic lungs were also reduced by quercetin. A substantial decrease of oxidative stress markers malondialdehyde (MDA) and 8-isoprostane was detected in lung samples of quercetin-treated silicotic mice. No effect was noted on the decreased levels of catalase (CAT). Increased lung resistance and elastance as well as airway hyper-reactivity to aerosolized methacholine were reduced by quercetin. No irritative effect was noted in the small intestine and colon in quercetin-treated animals. A similar inhibitory profile was noted after treatment of silicotic mice with N-acetylcysteine. Interestingly, in another set of experiments, we showed that quercetin significantly suppressed the proliferation of mouse lung fibroblast triggered by rmIL-13 *in vitro*. **Conclusion**: Our findings show that administration of quercetin effectively attenuated inflammation and fibrosis in the lungs of silica-challenged mice. This is an indicative that quercetin seems to constitute a promising therapeutic approach to be used in the case of chronic inflammatory lung diseases such as silicosis. **Financial support**: FIOCRUZ, CNPq, FAPERJ, CAPES and European Community (UE FP7- 2007-2013 - n° HEALTH-F4-2011-281608).

04.003 Increased TRPA1 mRNA expression and antioxidant enzymes activity may contribute to sex differences in pulmonary allergic inflammation in young mice prior exposed to ambient pollutant 1,2-naphthoquinone. Florenzano J, Santos KT, Feitosa KB, Soares AG, Rodrigues L, Teixeira SA, Muscará MN, Costa SKP ICB-USP – Farmacologia

Introduction: We have previously shown that neonate exposure to chemical ambient pollutant 1,2-NQ, found in diesel exhaust particle (DEP), enhanced susceptibility to lung allergic inflammation in male, but not in female mice, partially through up regulation of antioxidant enzyme activities. Moreover, recent evidence suggests that DEP, other combustion-derived particles and reactive oxygen species (ROS) can activate TRPA1 receptor, and then sensitizes vagal lung C-fibers, causing irritation and inflammation (Ruan et al., 2014, PLoS One., 3;9(4): e91763). **Aims**: This study was designed to investigate whether TRPA1 channels and an imbalance between the generation of oxidants compounds and the action of antioxidant defense system acts as one of the critical links between 1,2-NQ-induced increased differential susceptibility to allergic inflammation in male and female mice during puberty. **Methods**: Under approval of ICB/USP Animal Ethics Committee (113/07/CEEA), neonate male and female C57Bl/6 mice (2-5 g) were used and nebulized with 1,2-NQ (100 nM) or its vehicle for 3 alternate days. One group of animals were euthanized after 24 h of the last exposure to 1,2-NQ while the other group, sixteen and twenty three days later, were sensitized via subcutaneous (s.c.) route with a single dose of ovalbumin (OVA; 10 µg 0.2 ml^{-1} PBS) or vehicle. One group of animals on days 40, 41 and 42, were challenged with 10 mL of OVA 1% or its vehicle while the other group after two challenges with OVA, on day 42, were treated with TRPA1 antagonist (HC030031; 50mg/kg) 2 h before the challenge. The *in vitro* inflammatory parameters (e.g. leukocytes counts, levels of oxidants/antioxidants agents, and TRPA1 mRNA expression) in lungs were assessed after 24 h of the last challenge. Data are presented as mean ± SEM. Stats were performed by ANOVA followed by Bonferroni's test. $P<0.05$ was taken as significant. **Results**: The exposure to 1,2-NQ during the neonatal phase induced in neonate female mice an increase in the activity of antioxidant enzyme catalase while in the allergic young female mice, the pollutant enhanced antioxidant enzymes activities: catalase, GPx, GR and GST. Besides the increase in the activity of antioxidant system in the female lung, a significant increase of oxidative stress markers (e.g. protein expression of 3-NT, total activity of NOS and arginase) has also been shown in the lung of these animals. TRPA1 mRNA expression is higher in the lung of allergic young female mice prior exposed to 1,2-NQ compared to respective allergic females. Treatment of female with TRPA1 caused a significant decrease of total leukocytes in the BALF of allergic female mice, but not male, neonate exposure to 1,2-NQ when compared to female treated with the vehicle of the antagonist. **Conclusion**: We show, for the first time, that antioxidant activity is higher in the lung of young female, but not in male, mice prior exposed to 1,2-NQ during the neonatal period, and this seems to be correlated with high TRPA1 mRNA expression and reduced lung allergic inflammation compared to young male mice. **Acknowledgements**: Fapesp (2013/02115-0), CNPq and CAPES for financial support.

04.004 Corticosterone and Zymosan modulation of melatonin production in RAW 264.7 macrophage lineage. Silva DS, Almeida RKG, Pires-Lapa MA, Markus RP, Fernandes PACM IB-USP – Fisiologia

Introduction: Melatonin (MEL) is a hormone involved in the control of a number of physiological processes both in health and during defense responses. This hormone is produced during the night by the pineal gland in response to sympathetic activation (Fálcon, Prog Neurobiol, 58, 121, 1999). Besides the pineal, immune cells also produce MEL (Carrilo -Vico et al.,Curr Opin Investig Drugs,7, 423, 2006). The production of MEL by the pineal or immunocompetent cells is controlled by signals present during immunological responses (Markus et al., Neuroimmunomodulation, 14, 126, 2007). Glucocorticoids may potentiate or reduce the pineal synthesis of MEL depending on the pattern of adrenergic stimulation imposed to the gland (Fernandes et al., J Pineal Res. 41, 344, 2006). In macrophages, molecules associated with pathogens (LPS and Zymosan) and catecholamines induce the synthesis of MEL which, acting in an autocrine form, enhances phagocytosis of these cells (Muxel et al., 7 (12), PMC 35287221, 2012. Pires-Lapa et al., J Pineal Res, 55, 240, 2013). Objective: Considering the importance of the production of MEL in proper functioning of macrophages, the present study aimed the investigated the effects of corticosterone (Cort) on the production of MEL, induced or not by and Zymosan (Zy), in the murine RAW 264.7 macrophages lineage. **Methods**: Mouse macrophages RAW 264.7 cells were stimulated with Cort (1-300 nM, 3h), Zy (1µg / ml, 6 h) or Cort+Zy. The melatonin present in the culture media, was determined by ELISA KIT, and the expression of the enzymes responsible for melatonin synthesis, arylakylamine N-acetyltransferase (AA-NAT, phosporilated or not) and hidroxyindol-O-metiltransferase (HIOMT) were determined by immunocytochemistry. Data are expressed as mean ± sem; values of the immunocytochemistry were normalized by the mean fluorescence detected on respective control groups. **Results**: Cells incubated with 100 nM Cort showed increased production of MEL (control: 29.61±1.8 µg/mL vs Cort: 44.28±3.0 µg/mL; $p<0.05$, n=3-4), AA-NAT expression (control: 100.0±1.5% vs Cort: 147.3±1.7%; $p<0.01$, n=80) and HIOMT expression (control: 100.0±6.6% vs Cort: 427.5±18.2%; $p<0.01$, n=80). Zy increased melatonin production compared to cells stimulated with vehicle (vehicle: 46.76±2.8 µg/mL vs Zy: 100±8.2 µg/mL, $p < 0.01$, n=80) but, when the cells were incubated with Zy (1µg /ml) and Cort (1-100 nM), there was a dose-dependent decrease in the Zy-induced MEL production. Surprisingly, the cotreatment with Cort increased the expression of AA-NAT, and HIOMT compared to cells treatment only with Zy (AA-NAT - Zy: 149.9±4.1 % vs Cort 100: 165.6±5.0%, $p <0.05$; HIOMT – Zy: 253.1±19.0% vs Cort 100: 432.7±11.2%, $p<0.01$, n=80). However, when we analyzed pAA-NAT it was observed a decrease on its expression in cells treated with Cort+Zy compared to Zy group (Zy: 273.4±5.842% vs Zy+Cort100: 187.5±4.285%, $p< 0.01$, n=80).Conclusions: corticosterone and zymosan leverage melatonin production in macrophages increasing the expression of the key enzymes of the hormonal pathway, however, when both corticosterone and zymosan are present the production of melatonin and the expression of pAANAT is diminished. **Financial support**: CNPq-130846/2014-7, FAPESP-2013/13691-1

04.005 The mechanisms of NLRP3 and AIM2 inflammasome inhibition by flavonoids. Domiciano TP[1], Verri Jr WA[2], Jones HD[3], Chen S[4], Crother TC[4], Shimada K[4], Arditi M[4] – [1]UEL – Ciências da Saúde, [2]UEL – Patologia, [3]Cedars Sinai Medical Center – Pulmonary and Critical Care Medicine, [4]Cedars Sinai Medical Center – Pediatric, Infectious diseases and Immunology

Introduction: IL-1β is a highly inflammatory cytokine and significantly contributes to both acute and chronic inflammatory diseases. The secretion of IL-1β requires a unique protease caspase-1, which is activated by protein platforms called inflammasome. Accumulating evidences indicate a key role of reactive oxygen species (ROS) signaling for inflammasome activation during numerous inflammatory diseases. Flavonoids, constitute a group of naturally occurring polyphenolic molecules, attribute many biological activities to antioxidant effects. **Aims**: In this study we investigated the effect of three flavonoids, quercetin (QUC), naringenin (NRG) and silymarim (SIL) on inhibition of inflammasome activation. **Methods**: Bone marrow derived cells (BMDM) were primed with LPS (500 ng/mL) and 3 hr later followed ATP (5 mM), nigericin (10 µM) or alum (130 µg/mL) stimulation for NLRP3 inflammasome, dsDNA (400ng/ml) for AIM2 inflammasome, and *Salmonella Typhimurium* (MOI5) for NLRC4 inflammasome activation. BMDM were treated with QUC, NRG, SIL at the doses of 20 and 100 µM, 30 min before the signal-2 stimulation. We used ATG16l1$^{-/-}$ BMDM to observe inflammasome activation. In addition, NLRP3$^{A350/A350}$ BMDM primed with LPS, treated 1 hr after priming were used to observe quercetin inhibition on autoreactive Nlrp3 inflammasome. Supernatants were collected and assessed for IL-1β BY ELISA. The experimental protocol was approved by the Cedars-Sinai Medical Center Institutional Animal and Care and Use Committee guidelines (IACUC 4541). **Results**: We found that treatment with QUC, but not NRG and SIL, inhibited IL-1β secretion by NLRP3 and AIM2 inflammasome in a dose dependent manner. In addition, QUC inhibited autoreactive NLRP3 inflammasome. ROS also induces Autophagy process which can compensate cellular stress and inhibit NLRP3 inflammasome activation. To address this we used ATG16l1$^{-/-}$ BMDM to observe inflammasome activation. However, NLRP3 inflammasome inhibition by QUC was independent of autophagy as seen inhibited IL-1β secretion by QUC in ATG16l1 KO BMDM. Since QUC inhibited both NLRP3 and AIM2 as well as autoreactive NLRP3 inflammasome we assessed ASC speck formation to seek for a possible mechanism of the action. We observed that QUC reduced ASC speck formation by immunostaining and phosphorylation compared with the control. **Conclusion**: In conclusion, QUC can inhibit NLRP3 and AIM2 inflammasome activation and be a further potential therapeutic candidate for inflammasome associated inflammatory diseases. **Financial support**: Capes, CNPq 308052/2013-7 and MCTI/SETI/Fundação Araucaria and Parana State Government.

04.006 ADP treatment improves wound healing in diabetic mice. Borges PA[1], Brogliato AR[1], Figueiredo JB[1], Meyer-Fernandes JR[2], Neves SJ[1], Benjamim CF[1] [1]ICB-UFRJ – Farmacologia e Química Medicinal, [2]IBqM-UFRJ

Introduction and Aims: Chronics wounds are a health problem in worldwide, which affect 6.5 million patients in USA. Such problem in association with high global prevalence of diabetes, reflects the increase in diabetic ulcers. Considering the absence of an effective treatment for chronic wound, we investigated the possible beneficial effects of purinergic agonists in tissue repair. In the present work we explore the role of ADP in healing process of skin chronic wounds in diabetic mice. **Methods**: In this study, Diabetes Mellitus was induced by a single intravenous Alloxan injection (65 mg/Kg) 8 h after fast in Swiss male mice (20-24g). Mice were then separated in four groups and treated once a day per 14 days as following: a) control group: saline was topically applied on wound beds; b) ADP group, 30 μM of ADP was topically applied on wound beds; clopidogrel + saline: in which clopidogrel was given by gavage and saline was topically applied on wound beds; and clopidogrel + ADP, in which clopidogrel was given by gavage and ADP was topically applied on wound bed. Wounds contraction was measured at days 3, 7, 10, and 14 days after wounding. Wounds were collected at day 7, formalin fixed and paraffin-embedded. Skin sections were stained with HE (for granulation tissue), Sirius Red (for collagen), modified Sirius Red (for eosinophil), Alcian Blue (for mast cell), or immunostained for α-actin, laminin, macrophages, VEGF and TGF-α. Wounds were also collected at day 7 to perform MPO activity (for neutrophil indirect quantification), ELISA (TNF-α, IL-10 e IL-13) and CBA (IL-6, IL-10, MCP-1, IFN-γ, TNF, IL-12p70). **Results**: Our data showed that ADP was able to accelerate the wound healing of diabetic mice, which resembles the healing of non-diabetic mice. Clopidogrel treatment, a P2Y12 receptor antagonist, prevented the wound closure in diabetic mice treated with ADP. Interestingly, clopidogrel increased the wound size even in non-diabetic mice. Others nucleotides as adenosine, ATP, AMP5' and AMP3' at 30 μM did not accelerate the wound healing, as observed for ADP. Through histological analysis it was observed that ADP treatment improved the granulation tissue formation, collagen synthesis and increased the recruitment of eosinophils and neutrophils, and the population of mast cells on the seventh day. ADP stimulated the release of the cytokine IFN-γ on the third day and the IL-10 and IL-13 on the seventh day. In addition, at day 7, ADP was effective in increasing the differentiation of myofibroblasts and the expression of laminin, VEGF and TGF-α. Still in this time point, ADP seemed to increase the arginase^{+} cells and to reduce iNOS^{+} cells, which suggest the increase of M2 macrophages in the wound after ADP. **Conclusion**: Our results suggest that ADP accelerates wound healing in diabetic mice and the mechanism seems to be via recruitment of inflammatory cells to the wound, leading to an improvement of the wound repair. We expect that ADP may become a new treatment for chronic injuries. **Financial support**: CAPES, CNPq and FAPERJ. **IACUC approval**: 046/14.

04.007 Hypercorticosterolemia observed in diabetic rats depends on TLR4 activation. Magalhães NS[1], Torres RC[1], Prevatto JP[1], Gonçalves-de-Albuquerque CF[2], Martins MA[1], Silva PMR[1], Carvalho VF[1] – [1]Fiocruz – Farmacologia e Inflamação, [2]Fiocruz – Imunofarmacologia

Introduction: Diabetic patients and animals present a hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis and increase of glucocorticoid levels. Some diabetes-related morbidities are associated with an increase in glucocorticoids levels, including neuropathy and wound healing deficiency. Diabetic patients show high expression of TLR4 in monocytes as well as increased levels of DAMPs activators of these receptors in circulation. Activation of TLR4 in adrenal glands induces production and release of glucocorticoids. Therefore, in this study we investigated the role of TLR4 in the increasing of plasma glucocorticoid levels observed in diabetic rats. **Methodology**: The diabetes was induced by single injection (i.v.) of alloxan (40 or 65 mg/kg) into fasted male Wistar rats and C3H/He and C3H/HeJ mice, respectively. C3H/HeJ is a natural mutant mouse strain deficient for TLR4 signaling. All analyses were made 21 days after the beginning of the experiment. Circulating non-esterified fatty acids (NEFA) was measured by HPLC, while plasma corticosterone levels were quantified by radioimmunoassay. Furthermore, the expression of glucocorticoid receptors (GR and MR), ACTH receptor (MC2R) and TLR4 in adrenal and pituitary glands were assessed by immunohistochemistry. **Results and Discussion**: Diabetic rats presented high plasmatic levels of corticosterone, in parallel with reduced expression of GR and MR in pituitary and increased MC2R density in adrenal, compared to non-diabetic rats. Furthermore, diabetic rats showed high circulating amount of NEFA, including oleic acid and stearic acid, compared to non-diabetic rats (from 85 ± 3.6 and 68 ± 25.6 µM, mean ± SEM, n = 6, to 135 ± 20.2 and 142 ± 8.4 µM, mean ± SEM, n = 8; $P < 0.05$). In association of increased levels of NEFA activators of TLR4, diabetic rats showed high expression of TLR4 in both adrenal and pituitary glands. This data suggests a possible activation of TLR4 in adrenal and pituitary of diabetics, and this endogenous activation of this receptor in these glands could be responsible for the activation of HPA axis in diabetics. So, as a next step to support this hypothesis was induce diabetes in C3H/HeJ mice. 21 days after injection of alloxan, both control and TLR4 mutant mice strains presented hyperglycaemia and loss weight body, indicating that both strains developed diabetes. However, diabetic TLR4 mutant mice presented reduced levels of plasma corticosterone compared to diabetic control mice, suggesting that endogenous activation of TLR4 in pituitary and adrenal glands by DAMPs in diabetics induces a hyperactivation of HPA axis. **Keywords**: Diabetes, Glucocorticoids, HPA axis, NEFA and TRL-4. **Financial Support**: CNPq, FAPERJ and FIOCRUZ. **Animal Research Ethical Committee Approval**: Ethic Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation, License LW – 23/11.

04.008 Effect of gold nanoparticles on pulmonary inflammation caused by silica particles in mice. Ciambarella BT, Ribeiro NBS, Arantes ACS, Serra MF, Azevedo RB, Fernandes AJM, Martins MA, Silva PMR Fiocruz – Inflamação

Introduction: The inhalation of silica particles leads to development of silicosis, an occupational disease characterized by leukocyte infiltration, collagen deposition and granuloma formation. There is no efficient treatment available for fibrotic diseases, which demands the search for effective therapies to control silicosis. Remarkably, administration of gold nanoparticles (AuNPs) can lead to anti-inflammatory effects in different pathophysiological conditions. **Aims**: This study was undertaken to investigate the effect of aerosolization of AuNPs on lung inflammation and fibrosis trigerred by silica particles in mice. **Methods**: Anesthetized male Swiss-Webster mice received intranasal (i.n.) instillation of silica (10 mg/50 µL) or vehicle (saline). Treatment consisted of 3 aerosol administrations of AuNPs (6 and 60 µg/kg) on days 21, 24 and 27 after silica instillation. The analyses were made 24 h after the last administration and included the following parameters: i) lung function (resistance and elastance) and airways hyper-reactivity to metacholine (3 - 27 mg/mL) were measured by whole body invasive plesthysmography (Finepointe, Buxco System); ii) morphological alterations analyzed by histological techniques including staining with Hematoxylin-Eosin and Picrus sirius; iii) quantification of tissue collagen content and of nitric oxide (NO) done by Sircol and Griess techniques, respectively. All experimental procedures were approved by the Committee on Use of Laboratory Animals of Oswaldo Cruz Foundation (license LW 57/14). **Results**: Exposure of mice to silica particles yielded higher baseline lung resistance and elastance when compared to saline group. After aerosolization of the bronchoconstrictor methacholine, silica-provoked mice exhibited increased airway resistance and elastance. Therapeutical administration of AuNPs to silicotic mice inhibited lung function and hyper-reactivity, at both doses tested (6 and 60 µg/kg). Instillation of saline did not lead to abnormal deposition of collagen, though mice challenged with silica showed a massive increase in extracellular matrix deposition as revealed by histology and collagen quantification. AuNPs significantly reduced granuloma formation and collagen deposition, only at the lower dose tested. The increased levels of tissue NO were not modified after AuNP treatment. **Conclusion**: Our findings show that aerosolized AUNPs effectively inhibited lung function alteration and airways hyper-reactivity in silicotic mice, though fibrosis was suppressed by the lower dose. Additional studies are needed to characterize better the mechanism involved in the suppressive effect of AuNPs on the experimental model of silicosis in mice. **Financial support**: PAPES6/FIOCRUZ, CNPq, FAPERJ, CAPES and European Community (UE FP7- 2007-2013 - n°HEALTH-F4-2011-281608).

04.009 JM25-1, a lidocaine analogue combining airway relaxant, anti-inflammatory and antieosinophilic properties: **implications for new asthma therapy.** Cotias AC[1], Serra MF[1], Neves JS[2], Couto GC[1], Pão CRR[1], Olsen PC[2], Anjos-Valotta EA[1], Faria RX[3], Costa JC[3], Cordeiro RSB[1], Carvalho KIM[1], Silva PMR[1], Martins MA[1] [1]Fiocruz – Fisiologia e Farmacodinâmica, [2]UFRJ, [3]Fiocruz

Background: Eosinophils are presumed to play a central role in the pathogenesis of allergic diseases such as asthma. The anesthetic lidocaine inhibits cytokine-induced eosinophil survival and improved asthma features in animal models and human patients, but adverse effects related to local anesthesia limit its efficacy. This study investigated the effect of a lidocaine analogue, JM25-1, screened for reduced anesthetic activity, upon human eosinophil activation/survival and smooth muscle contraction *in vitro*. Translation to an animal model of asthma was also explored **Methods**: Sodium currents were evaluated using patch clamp of GH3 cells. We used human blood eosinophils for studies of p38 phosphorylation and apoptosis using western blotting and flow cytometry, respectively. Effects on tracheal smooth muscle contraction were studied in conventional organ baths. Studies on functional and inflammatory changes were done in ovalbumin-sensitized mice, assessing lung function, peribronchiolar eosinophil density, mucus production, extracellular-matrix deposition, inflammatory mediators and GATA-3 levels. License number for this study is LW23/10. **Results**: Carbachol- and calcium-induced tracheal contractions were sensitive to 1 mM JM25-1 [Emax inhibition (%): 67 ± 10 with JM25-1 vs. 41 ± 11 with lidocaine ($P<0.06$) for carbachol; 100 ± 3 with JM25-1 vs. 36 ± 26 with lidocaine ($P<0.02$) for calcium; mean ± SD; n=9 each], despite the lower potency to inhibit Na+ current (IC50=152 ± 5 vs. 0.2 ± 5 mM with lidocaine; n=6; $P<0.01$). JM25-1 (0.3 mM) also inhibited eosinophil survival [dead cells (%): 2.3 ± 0.5 to 8.4 ± 1; n=5; $P<0.06$)] and lymphocyte proliferation [cells in phase S+G2 (%): 28 ± 3 to 10 ± 1; n=6; ($P<0.06$)]. Nebulized JM25-1 decreased eosinophil and neutrophil numbers from 12 ± 2 to 3 ± 1 x 104/µm2 (n=6; $P<0.001$) and from 2 ± 0.3 to 0.4 ± 0.3 x 104/µm2 (n=6; $P<0.001$), respectively. Other parameters, including airway hyper-reactivity, cytokines, mucus and extracellular matrix deposition were also sensitive to JM25-1. **Conclusions**: These findings demonstrate the anti-asthma properties of JM25-1, pointing out its putative value in drug development for asthma and other airway-spasmodic disorders. **Financial support**: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Instituto Nacional de Ciência e Tecnologia – INOFAR (INCT-INOFAR), European Community's Seventh Framework Programme [FP7-2007-2013] under grant agreement n°HEALTH-F4-2011-281608.

04.010 Annexin A1 (ANXA-1)-mimetic peptide controls the inflammatory and fibrotic effects induced by house dust mite (HDM) in mice. Ferreira TPT[1], Souza ET[1], Trentin PG[1], Silva TV[1], Castro GC[1], Arantes ACS[1], Flower R[2], Perretti M[2], Martins MA[1], Silva PMR[1] – [1]Fiocruz, [2]WHRI – Biochemical Pharmacology

Introduction: Asthma is an airway inflammatory response, driven by Th2 cells, marked by eosinophilic infiltration, bronchial hyper-reactivity, mucus exacerbation and peribronchial fibrosis. Endogenous glucocorticoid hormones are critical on their potent anti-inflammatory activity, a response partially dependent on the release of pro-resolving mediators such as AnxA1. This protein is shown to be secreted in respiratory fluid and reported to be up-regulated in asthmatic bronchial lavage fluid. In many inflammatory and cellular settings, the anti-inflammatory activity of AnxA1 is reproduced by peptides derived from the N-terminal region of the protein, including the peptide Ac2-26. **Aim**: In this study we investigated the therapeutic properties of the N-terminal AnxA1-derived peptide Ac2-26 on experimental model of asthma induced by HDM in mice. **Methods**: AnxA1 null and wild type littermate (Balb/c) mice were sensitized with intranasal instillation of house dust mite (HDM - 25 µg/25 µL), every other day, during 3 weeks. In another set of experiments, wild type littermates were treated therapeutically with intranasal peptide Ac2-26 (200 µg/mouse) or budesonide (10µg/mouse), 1 h before antigen, starting on the week 2 of sensitization. Twenty four hours after the last challenge, lung function, inflammatory and fibrotic markers were measured. All the experimental procedures were approved by the Ethics Committee of Animal Use of FIOCRUZ (license LW57/14). **Results**: We found that HDM led to increased airways hyper-reactivity to methacholine and intense infiltration of leukocytes in the BALF. A marked eosinophil accumulation was noted in the peribronchial area as well as an excessive deposition of extracellular matrix. Increased tissue generation of inflammatory and fibrotic cytokines (IL-4, TGF-beta, eotaxin -1 and -2 and MCP-1) was also detected. A clear exacerbation of these pathological changes was observed in AnxA1 null mice as compared to the wild type littermate controls. Intranasal peptide inhibited HDM-induced airway hyper-reactivity and accumulation of leukocytes in the BALF. Ac2-26 also prevented other pathophysiological changes triggered by HDM in lung tissue including peribronchial eosinophil and neutrophil infiltration, subepithelial fibrosis, increased content of mucus and levels of cytokines. Treatment with budesonide was able to afford an inhibitory effect of HDM-induced lung function and morphological alterations, though being less effective that the peptide ac2-26 in some parameters. **Conclusions**: Taken together, our findings show that AnxA1 null mice show an exacerbation of several aspects of asthma, indicating that AnxA1 plays a pivotal role in the negative regulation of features of severe asthma. In addition, The AnxA1-derived peptide Ac2-26 protects against several pathological changes associated with allergen provocation in wild-type mice, suggesting a pharmacological correlation that turns possible the development of a therapeutic agent for severe asthma. **Financial support**: FIOCRUZ**,** CNPq, FAPERJ (BR) and European Community (UE FP7- 2007-2013 - n°HEALTH-F4-2011-281608).

04.011 SN-38, the active metabolite of the anticancer agent irinotecan, is an antagonist of the toll-like receptor 4. Wong DVT[2,1], González RH[2], Wanderley CWS[2], Borges VF[3], Leite CAVG[2], Batista GLP[2], Ribeiro-Filho HV[2], Lima JB[3], Bem AXC[2], Silva KO[1,2], Brito GAC[4], Cunha TM[3], Lima-Júnior RCP[2], Cunha FQ[3], Ribeiro RA[2,1] [1]ICC, [2]UFC – Fisiologia e Farmacologia, [3]FMRP-USP – Farmacologia, [4]UFC – Morfologia

Introduction: Severe diarrhea (15-25% of patients) and the associated intestinal mucositis (IM) are common side effects of colorectal anticancer therapy with irinotecan (IRI). We have demonstrated the role of TNF-α, IL-1β, IL-18, IL-33 and nitric oxide in the pathogenesis of IRI-induced intestinal mucositis. Furthermore, IRI significantly damage the gastrointestinal tract, which is associated with bacterial translocation to peripheral organs in a process that is dependent on toll-like receptors 2 and 9 (TLR2 and TLR9). However, the role of toll-like receptor 4 (TLR4) in the pathogenesis of IRI-induced IM is not completely understood. **Aims**: this study aimed to evaluate the involvement of TLR4 in the pathogenesis of IRI-associated IM. **Methods**: C57BL/6 (WT) mice (20-24g, n=6-7) and TLR4 ($TLR4^{-/-}$) knockout animals were given either saline or IRI (45 mg/kg i.p/4 days). On day 7, weight loss, diarrhea and blood leukocyte were assessed. Following euthanasia, ileum samples were obtained for myeloperoxidase (MPO) assay, morphometric analysis, *Cox-2, Il-18* and *PI3k* gene expression by (q)RT-PCR. Raw-Luc macrophage cell line (transfected with NF-κB luciferase promoter) was used to investigate the role of SN-38, the IRI active metabolite, in the regulation of TLR4 signaling. These cells were stimulated with LPS (100 ng/mL), SN38 (0.2; 2 e 20 µM), LPS + SN-38 or Irinotecan (50 µM). The interaction of SN-38 with TLR4 was also investigated through molecular docking. Kruskal Wallis/Dunn's test or ANOVA/Bonferroni's test were used for statistical analysis. $P<0.05$ was accepted. **Results**: Irinotecan induced weight loss, diarrhea, leukopenia, increased MPO activity, morphometric alterations, as well as increased expression of *Cox-2* and *Il-18,* and decreased expression of *Pi3k* in intestinal samples of WT animals versus saline-injected group ($P<0.05$). Additionally, $TLR4^{-/-}$ mice that were injected with IRI showed a more severe ($P<0.05$) weight loss, diarrhea (2[1-3]), intense neutrophil infiltration (1745±346), decreased villus/crypt ratio (1.63±0.07), increased *Il-18* expression (1.19±0.09) when compared with IRI-injected WT mice (diarrhea: 0[0-1]; neutrophil infiltration: 678±260; *Il-18*: 0.63±0.09; $P<0.05$). Furthermore, LPS (100ng/mL) markedly enhanced the relative luciferase activity in Raw-Luc cells (8.70±0.7), which was significantly inhibited by 2 µM of SN-38 (5.52±0.6). The inhibitory effect of SN-38 was completely abrogated by growing concentrations of LPS ($P<0.05$). Moreover, the molecular docking assay suggested that SN-38 interacts with the hydrophobic channel of MD-2/TLR4 protein complex in the same biding site of LPS. Conclusions: This study suggests that SN-38 acts as a TLR4 antagonist leading to a pro-inflammatory response during IRI-induced intestinal mucositis. Furthermore, our findings suggest that TLR4 seems to protect mice from the development of IM. **Financial support**: CNPq/CAPES/FUNCAP. (CEPA 99/10).

04.012 Evaluation of the TLR7 partial agonist TMX-302 as anti-inflammatory and antiasthmatic agent in murine models of lung respiratory diseases. Ghilosso-Bortolini R[1], Ferreira TP[1], Arantes AC[1], Silva PMR[1], Maj R[2], Martins MA[1] [1]Fiocruz – Farmacologia e Inflamação, [2]Telormedix SA

Introduction: Prior studies have demonstrated the anti-inflammatory properties of repeated application of low doses of TLR7 agonists. Additionally, modified adenine inhibits Th2-mediated murine lung inflammation by triggering TLR7. **Aim**: The current study was undertaken in order to investigate the effect of TMX-302, a PEGylated purine-like compound characterized by TLR7 partial agonistic activity, on murine models of acute lung injury (ALI) and asthma. **Methods**: Naïve and ovalbumin-sensitized A/J mice were subjected to a single intranasal challenge of LPS and two consecutive daily challenges of ovalbumin (OVA) (25 µg/25 µL), respectively. The readouts were done at 18 h post-LPS and 24 h after the last OVA provocation. Airway hyper-reactivity, total and differential leukocytes in bronchoalveolar lavage fluid (BALF) and lung tissue, plasma leakage and pro-inflammatory cytokines were quantified. TMX-302 was administered subcutaneously (s.c.) 24 h and 1 h before LPS and 1 h before OVA. TMX-302 was also topically delivered through nebulizations or nasal instillations done at 1 h before allergen challenges. **Results**: As expected, both LPS and allergic stimuli yielded states of airway hyper-reactivity (AHR) to methacholine accompanied by intense lung inflammatory responses, which were characterized by plasma leakage and neutrophilic and eosinophilic infiltrates after LPS and allergen, respectively. TMX-302 (500 nmol/mouse, sc) abolished LPS-induced AHR and plasma leakage, and attenuated (50%, $P<0.05$) the neutrophil accumulation. This treatment also inhibited allergen-induced AHR and eosinophil accumulation (75%, $P<0.05$), in association with significant decreases in the levels of lung eotaxin-1 and -2, MIP-1α and KC. Aerolized TMX-302 (6% for 30 min) also inhibited allergen-induced AHR, under conditions where the nasal instillation of TMX-302 (63.5 nmol/mouse) was inactive, differently from to what was observed following dexamethasone. **Discussion**: As administered subcutaneously, TMX-302 prevents inflammatory changes as well as AHR in murine models of acute asthma and ALI. Allergen-induced AHR was also sensitive to aerolized but not intranasal instillation of TMX-302 at the dosages applied. Altogether, these findings show the anti-inflammatory potential of TMX-302 pointing out its value in drug discovery for clinical conditions, such as asthma, where there is lung inflammation and bronchospasm. Further studies on intranasal delivery and formulations aspects should be conducted, concerning the efficacy and safety of TMX-302 delivered via that route. **Financial support**: FIOCRUZ, CNPq, EC-FP7/TIMER consortium, Animal Research Ethical Committee approval at Fiocruz (CEUA-FIOCRUZ L-034/09).

04.013 The absence of the atypical chemokine receptor D6 leads to high mortality during sepsis. Castanheira FVS, Sonego F, Kanashiro A, Borges VF, Colon DF, Donate PB, Melo PH, Russo RC, Amaral FA, Teixeira MM, Graham GJ, Locati M, Cunha TM, Alves-Filho JC, Cunha FQ USP – Farmacologia

Introduction: Neutrophils are the first cell line to reach the primary focus of an infection and chemokines have a fundamental role in this process. During sepsis, chemokines also contribute to the neutrophil infiltration to remote organs and to multiple organ failure. Under normal physiological conditions neutrophils do not express the CC chemokine receptor subfamily and, as consequence, do not respond to CC chemokines. However, our group has shown that during sepsis neutrophils become responsive to these chemokines and express CC chemokine receptors. Recently, a new chemokine receptor named D6 has been studied and it has been described as an atypical receptor due to its involvement in the removal and degradation of CC inflammatory chemokines. **Aim**: to investigate the involvement of D6 in sepsis. **Methods**: All experiments were performed according to our institution's ethical guidelines (169/2011). Sepsis was induced in C57BL/6 and D6 deficient mice ($D6^{-/-}$) by cecal ligation and puncture (CLP). Markers of organ damage, neutrophil infiltration, chemokine levels on remote organs and D6 expression were determined 24 hours after CLP. The survival rate was also assessed. The means of the parameters evaluated in WT and $D6^{-/-}$ mice submitted to CLP were analyzed by ANOVA, followed by Bonferroni test, or by t test and the survival rate by the Mantel-Cox log rank test. **Results**: We observed that D6 expression is modulated after sepsis induction in remote organs, increasing in the lung (Sham: 1.0± 0.06; Sub-letal CLP: 2.9±0.07), heart (Sham: 1.0±0.06; Sub-letal CLP: 2.4±0.3) and kidney (Sham: 1.0± 0.06; Sub-letal CLP: 1.7±0.2). Interestingly, the increasing in D6 expression in these organs occurred only after sub-letal, but not letal sepsis induction. Associated with this, we showed increased CC chemokines levels and neutrophil infiltration, assessed by myeloperoxidase (MPO), in the lung (MPO- WT: 15120±502.3 and $D6^{-/-}$: 19350±807.4; CCL3- WT: 791.2±289.2 and $D6^{-/-}$: 2864±487.3), heart (MPO- WT: 119.8±25.8 and $D6^{-/-}$: 208.2±19.7; CCL2- WT: 656.8±60.8 and $D6^{-/-}$: 837.5±47.2; CCL3- WT: 34.82±20.8 and $D6^{-/-}$: 731.4±284.5) and kidney (CCL5- WT: 785.2±142.7 and $D6^{-/-}$: 1717±204.5) of $D6^{-/-}$ mice after sub-letal CLP induction. These mice also showed higher levels of biochemical markers of lesion in the heart (CK-MB; WT: 376.5±34.1 and $D6^{-/-}$: 554.8±75.2), kidney (BUN; WT: 59.7±13.4 and $D6^{-/-}$: 116.3±23.4) and liver (TGO; WT: 117.5±2.9 and $D6^{-/-}$: 230.4±33.7) then WT animals. As consequence, $D6^{-/-}$ mice exhibited a significant reduction in the survival rate, as compared to WT animals (WT: 88% and $D6^{-/-}$: 33%). In conclusion, our data indicate that D6 has a protective role during sepsis, mediating the reduction of chemokine levels in remote organs and, consequently, the reduction of organ damage. **Financial support**: CNPq, FAPESP, CAPES, FAEPA

04.014 Effects of Resolvin D1 on the allergic eosinophilic inflammation in obese mice. Tavares EBG, Calixto MC, André DM, Antunes E FCM-Unicamp – Farmacologia

Introduction: In the course of the inflammatory process, the essential ω-3 acid docosahexaenoic acid (DHA; C22: 6) suffer enzymatic transformations yielding anti-inflammatory and pro-resolution mediators, including the series D resolvins (1). DHA-derived mediators such as resolvin D1 (RvD1) inhibit neutrophil activation, regulate cytokine production, and decrease eosinophil (EO) recruitment and activation (2, 3). Obesity increases asthma severity and reduce the efficacy of standard medications for this disease (4). High-fat diet (HFD)-induced obesity enhances the pulmonary eosinophilic inflammation in a murine model of allergic disease (5). In this study we have hypothesized that RvD1 has anti-inflammatory and pro-resolving actions in ovalbumin (OVA)-challenged obese mice. To achieve this, we evaluated the EO infiltration in bronchoalveolar lavage fluid (BALF) and lung tissues in HFD obese mice sensitized and challenged with OVA as compared with control non-obese (lean) mice. **Methodology**: Male C57BL/6J mice received standard-chow diet (control group) or high-fat diet (obese group) for 12 weeks. Mice were sensitized with OVA (100 µg, s.c) on days 0 and 7. On days 14, 15, 16 and 17 mice were intranasally challenged with OVA (10 µg). Control and obese mice received RvD1 (5 µg/Kg, i.p.) 1h before each OVA challenge. Analysis of cell infiltration in BALF and lung histology was performed 24 h after the last OVA challenge. Data were expressed as means ± SEM for 6 to 9 mice in each group. ANOVA followed by Tukey test was used to statistically analyze data, and $P<0.05$ was accepted as significant. **Results**: The EO number in BALF was significantly lower in OVA-challenged obese compared with lean group ($P<0.05$), as previously described (5). Treatment with RvD1 in lean group reduced by 53% ($P<0.05$) the EO number in BALF. The EO infiltration in peribronchiolar and perivascular regions of lean mice was also significantly reduced by RvD1 treatment (42% and 70% reductions, respectively; $P<0.05$). In obese group, RvD1 treatment significantly increased the EO number in BALF and peribronchiolar regions, but rather reduced the EO counts in perivascular regions (59 ± 6.2 and 17 ± 1.5 cells/mm^2, respectively; $P<0.05$). Levels of cytokine IL-10 and eotaxin in BALF in obese mice were decreased by RvD1 treatment ($P<0.05$). **Conclusions**: In lean mice, RvD1 treatment decreases the allergic lung eosinophilic, demonstrating a protective anti-inflammatory action. In obese mice, our findings that RvD1 reduces EO counts in perivascular regions concomitantly with increases in peribronchiolar regions and BALF suggest that RvD1 acts to accelerate the EO transit from pulmonary microvasculature to airway lumen. **Financial support**: CNPq. The experimental protocols have been approved by the Ethics Committee of UNICAMP (N$^{o.}$ 3493-1). **References**: 1. Serhan CN et al. J. Exp. Med. 196: 1025; 2002. 2. Recchiuti A et al. J. Biol. Chem. 282: 9323; 2011. 3. Alexandre RP et al. J. Immunol. 189: 1983; 2012. 4. Peters-Golden M et al. Eur. Respir. J 27: 495; 2006. 5. Calixto MC et al. Br. J. Pharmacol. 159: 617; 2010.

04.015 Identifying macrophages autophagy phenotypes in diabetes. Sunahara KKS[1], Nunes FPB[2], Sannomya P[3], Martins JO[2] [1]FMUSP – Fisiopatologia, [2]FCF-USP – Análises Clínicas e Toxicológicas, [3]FMUSP

Introduction: Diabetes mellitus (DM) is characterized by hyperglycemia, associated to a lack or inefficiency of the insulin to regulate glucose metabolism. DM is also marked by alterations in a diversity of cellular processes that need to be further unraveled. **Aims**: We examined the autophagy pathway in diabetic rat macrophages before and after treatment with insulin. **Methods**: To investigate the autophagy pathway, microtubule-associated protein light-chain (LC) 3 and autophagy protein (Atg) 12 were used. **Results**: Insulin exerted antagonistic effects on macrophages from different tissues. Macrophages from bronchoalveolar lavage (BAL) enhanced their LC3 autophagosome bound content after treatment with insulin whereas splenic macrophages from red pulp in diabetic rats failed to enhance their Atg 12 levels compared with control animals. Insulin treatment in diabetic rats did not change LC3 content from bone marrow-derived macrophages (BMM). M1 and M2 macrophages behaved accordingly to the host they were derived from. Diabetic M1 from BMM had their LC3 vesicle-bound content diminished and M2 from BMM enhanced their LC3 levels and insulin treatment failed to rescue autophagy to the control levels. The Insulin normalized CINC-2 level but did not modulate the autophagy markers. **Conclusions**: Taking together, diabetic macrophages derived from different compartments show different levels of autophagy markers compared with healthy animals, therefore, they suffer differently in the absence of insulin. **Financial support**: FAPESP (2010/02272-0 and 2014/05214-1); CNPq (470523/2013-1) and CAPES. **Keywords**: Insulin, Diabetes Mellitus, Alveolar macrophages, Innate immunity, Autophagy. **References**: 1. Levine B, Mizushima N, Virgin HW: Autophagy in immunity and inflammation. Nature, 469: 323-35, 2011. 2. Sugawara I, Mizuno S: Higher susceptibility of type 1 diabetic rats to Mycobacterium tuberculosis infection. J Exp Med, 216: 363-70, 2008. 3. Bonilla DL, Bhattacharya A, Sha Y, Xu Y, Xiang Q, Kan A, Jagannath C, Komatsu M, Eissa NT: Autophagy regulates phagocytosis by modulating the expression of scavenger receptors. Immunity, 39: 537-47, 2013. 4. Deretic V, Saitoh T, Akira S: Autophagy in infection, inflammation and immunity. Nat Rev Immunol, 13: 722-37, 2013. 5. Rabinowitz JD, White E: Autophagy and metabolism. Science, 330: 1344-8, 2010.

04.016 Anti-inflammatory activity of tyrosol salicylate derivatives. Aguiar RP[1], Wiirzler LAM[1], Silva-Comar FMS[1], Rodrigues PJ[1], Cardia GFE[1], Silva-Filho SE[1], Uchida NS[1], Rocha BA[1], Velázquez-Martínez CA[2], Cuman RKN[1] [1]UEM – Farmacologia, [2]University of Alberta – Ciências Farmacêuticas

Introduction: This research involves the chemical modification of polyphenols, Tyrosol specifically, trying to increase their potency *in vitro* and in vivo. The first chemical modification of these new drug prototypes it was the **Introduction**: of a carboxylic acid group adjacent to one of the phenol groups present in these compounds, which creates a new salicylate moiety (Derivative 1). Acetylation of phenol groups in these molecules produces acetylsalicylate derivatives (Derivative 2), which may be regarded as aspirin-like natural polyphenols. The rationale behind this modifications is to transform natural polyphenols into acetylating drugs expecting to potentiate their natural anti-inflammatory properties. **Methods**: The MTT assay, a colorimetric assay for assessing cell viability, it was used in concentrations from Tyrosol, Derivatives 1 and 2 of 10, 30, 60 and 90 µg/ml, with neutrophils cellular line. Anti-inflammatory activity was evaluated by paw edema model induced by carrageenan (Cg - 500 µg/paw) in Balb/c mice. The same volume of vehicle (0.9% saline) was injected into the contralateral paw. The volume of paws was determined at times of 60, 120, 240 and 360 minutes after Cg application, with a plethysmograph apparatus. The increase of final volume of the paw was calculated by subtracting the volume of the paw injected with saline (control paw) by volume of the paw injected with Cg. Mice (n=6) were treated orally, one hour before Cg injection with Tyrsol, Derivatives 1 and 2 at doses of 50 and 100 mg/Kg or indomethacin (Indo) at a dose 20 mg/Kg. The results were statistically analyzed using ANOVA followed by Tukey's test. Differences were considered significant at $P<0,05$. **Results**: Our data indicated that Tyrosol, Derivative 1 and 2 did not present *in vitro* cytotoxicity at any of the concentrations tested. The Cg injection increased paw edema at 1^{st}, 2^{nd}, 4^{th} and 6^{th} hours (3.3 ± 0.3; 5.6 ± 0.5; 7.5 ± 0.6 and 9.5 ± 0.4, respectively). Tyrosol and Derivative 1 treatment at doses of 100 mg/Kg caused a significant reduction at 6^{th} hours after Cg injection (Tyrosol = 29.5%; Derivative 1 = 23.7%). On the other hand, Derivative 2 treatment at a dose of 100 mg/Kg caused a significant reduction at 4^{nd} and 6^{th} hours after the Cg injection (Derivative 2 = 28.4% and 26.4%, respectively). Indo treatment at the dose of 20 mg/Kg caused a significant reduction at 2^{nd}, 4^{th} and 6^{th} hours after Cg injection (Indo = 64.2%, 76% and 76.8%, respectively). **Conclusion**: The results showed that treatment with Tyrosol and Derivatives 1 and 2 obtained similar inhibitory effects on the development of paw edema, but the acetylation improve just a little bit the activity from derivatives in this model. Thus, the data provide evidence that the molecular modification improved the properties of Tyrosol. **Sources of research support**: CAPES/CnpQ; Fundação Araucária-PR. The experimental protocol was approved by the Ethics Committee on Animal Experimentation of the State University of Maringa (CEAE/UEM 008/2015).

04.017 Anti-inflammatory and anti-nociceptive effects of quercetin in a chronic model of titanium dioxide (TIO_2)-induced arthritis in mice. Borghi SM[2,1], Mizokami SS[1], Pinho-Ribeiro FA[1], Casagrande R[3], Verri Jr WA[1] [1]UEL – Ciências Patólogicas, [2]UEL – Patologia, [3]UEL – Ciências Farmacêuticas

Introduction: Arthritis affects individuals of different age groups and your progression is characterized by serious injuries in joints surfaces. In some cases, replacements of the original tissues may be necessary. Biomaterials like titanium dioxide (TiO_2) are often used for making orthopedic prosthesis, and a constant concern about complications that accompanying the use of biomaterials in implants consider the release of debris originated as prosthesis initiates the wear process. Moreover, previous studies demonstrated that TiO_2 induces inflammation in different experimental conditions. The flavonoid quercetin, a phenolic compound present in plants, and foods that comprises human diet, is known to present several biological activities like antinociceptive, anti-inflammatory and antioxidant effects. In this sense, its possible use in the treatment of TiO_2-induced arthritis should be considered. **Aims**: Evaluate the analgesic and anti-inflammatory effects of quercetin on TiO_2-induced arthritis model in mice. **Methods**: Mice were treated with quercetin (10-100 mg/kg, i.p.) or vehicle (DMSO) for 30 days after intra-articular administration of TiO_2 (0.1-3 mg, right knee), and mechanical hyperalgesia (electronic von Frey apparatus), edema (paquimeter), histopathological changes (H&E staining), leukocyte recruitment to the synovial tissue (total and differential cell count), proteoglycan degradation (cinetic-colorimetric assay), cytokine production (ELISA), COX-2, bone resorption and inflammasome activity (the last three by RT-qPCR) were evaluated. **Results**: Quercetin treatment dose-dependently inhibited articular mechanical hyperalgesia, edema, histophatological lesions and leukocyte influx to the synovia induced by intra-articular injection of TiO_2. Regarding the analgesic and anti-inflammatory mechanism of quercetin, it was demonstrated the inhibition of TiO_2-induced proteoglycan degradation, cytokine production (TNF-α, IL-1β, IL-6 and IL-10), COX-2 expression, bone resorption and inflammasome activity. Furthermore, quercetin treatment activates Nrf2/HO-1 signaling pathway in knee joint samples. Importantly, quercetin does not induced stomach, liver or kidney damage in the tested doses. **Conclusions**: These results evidenced that quercetin possesses analgesic and anti-inflammatory effects in TiO_2-induced arthritis, showing up as a potent therapeutic / prophylactic compound to control prosthesis wear process-related complications. **Acknowledgements and financial support**: We appreciated the technical support of Dr. Jefferson Crespigio. This work received financial support from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Ministério da Ciência, Tecnologia e Inovação (MCTI), Secretaria da Ciência, Tecnologia e Ensino Superior (SETI), Fundação Araucária and Governo do Estado do Paraná. **Ethical approval**: Animals' care and handling procedures were in accordance with the International Association for Study of Pain (IASP) guidelines and with the approval of the Institutional Ethics Committee for Animal Research of the Universidade Estadual de Londrina, process number 4480.2014.48.

04.018 Modulation of pathways of the resolution of inflammation following hydroalcoholic crude extract from *Casearia sylvestris* (HCE-CS) application in experimental complex regional pain syndrome –Type I (CRPS-I). Piovezan AP[1,3,2], Batisti AP[3], Benevides MLACS[3], Lenfers BT[4], Fausto LSL[3], Martins DF[3], Seed M[2], Headland SE[2], Cooper D[2], Souza PS[2], Perretti M[2] [1]PPGCS, [2]WHRI, [3]LaNex-Unisul, [4]LaNDI-UFSC

Introduction: HCE-CS possesses anti-inflammatory action in the carrageenin-induced paw edema model, with evidence indicating modulation of neutrophil reactivity. In an animal model of CRPS-I, the lipoxin A_4 analogue BML-111 and HCE-CS reduced edema and mechanical allodynia to a similar degree. **Aims**: Establish if formyl-peptide receptor type 2 (FPR2) mediates the effects of BML-111 and HCE-CS in this model. Correlated macroscopic effects with histological alterations in the paws. **Methods**: Male Swiss mice (ca. 30g) were anesthetized and submitted to ischemia of the right hind paw with a tourniquet (3h), then reperfusion (RE) was allowed. Different groups of animals were pre-treated with vehicle (saline, 10ml/kg s.c.) or the FPR2 antagonist (WRW4, 10µg s.c. per animal) and 15 min later received vehicle, BML-111 (1µg/animal, s.c) or HCE-CS (30mg/kg, orally): these pharmacological treatments were applied 24h or 48h after RE. Edema was measured as changes in paw thickness (in µm) and mechanical allodynia was assessed by paw withdrawal in response to 10 applications (%) of von Frey filament (0.4 g). For histological analyses on paraffin embedded blocks from mice paws harvested at 49 h after RE, sections were obtained (5 µm) and stained using H&E technique. Color images from metatarsal region were captured and amplified using ImagePro Plus®: the number of immune cells infiltrating the skeletal muscle (same size/area applied to all pictures, average of 3 images for animal) were counted with tag points marker. All protocols were approved under register of the CEUA-UNISUL (13.036.4.03.IV). In all cases, data are presented as mean±SEM (n=6-12), One or Two way ANOVA followed by Dunnet or Bonferroni, respectively ($p \leq 0.05$). **Results**: While no substantial effects were observed on edema (not shown), the FPR2 antagonist WRW4 abrogated the protective action of HCE-CS (from 23±8% vehicle to 56±9% for the extract) and BML-111 (from 22±10% vehicle to 63±12% for the small molecule) against mechanical allodynia in CRPS-I model, bringing values back to those of the vehicle groups. Induction of CRPS-I into the right hind paw of the mice provoked an important infiltration of cells (61.3±5.4 cells) that surrounded skeletal muscle of the limb when compared to the control group (19.5±1.6 cells), as monitored histologically. This cellular process was not affected by BML-111 (49.7±3.6 cells) or HCE-CS (61.2±6.1 cells). **Conclusion**: These data indicate a modulation of resolution pathways (possibly centred on the AnxA1-LXA_4-FPR2 circuit) following HCE-CS administration: these effects are selectively operative in allodynia and are independent from reduction of cell infiltration. **Financial support**: CNPq, FAPESC, UNISUL (Brazil) and WHRI-ACADEMY (UK). “Research leading to these results has received funding from the people Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme (FP7/2007-2013) under REA grant agreement n° 608765”. Contents reflect only the author’s views and not the views of the European Commission. APP e MP are Co-funded by Marie Curie Actions.

04.019 Influence of leptin receptor expression in lipid mediators production, in primary culture of pulmonary endothelial cells from intrauterine undernourished rats, stimulated by LPS. Azevedo GA[1], Balbino AM[1], Santos LA[1], Gil NL[1], Silva MM[1], Fernandes L[1], Landgraf MA[2,1], Landgraf RG[1] [1]Unifesp-Diadema – Inflamação e Farmacologia Vascular, [2]USP – Farmacologia

Introduction: Intrauterine undernourishment can induce a range of fetal adaptations, which can lead to permanent alterations in adult life, such as decreased lung function and reduced lung allergic inflammation. The vascular endothelium is closely related with the circulatory control, and plays an important role in cellular and molecular events which occur during immune system reactions and tissue injuries. Leptin, a hormone mainly synthesized by adipose tissue, influences a wide variety of biological functions, including hematopoiesis, angiogenesis, phagocytic activity and reactive oxygen species production, endothelial cell activation and production of Th1 cytokines in T cells. **Objectives**: To evaluate the expression of long-form leptin receptor in rat pulmonary endothelial cells intrauterine malnutrition and your role in the production of lipid mediators. **Methods and Results**: Pulmonary endothelial cells were obtained from rats at 12 weeks age, submitted or not to global intrauterine malnourishment (50% reduction) throughout the gestational period. These cells were stimulated with leptin (10ng/mL) or LPS (1µg/mL) or leptin plus LPS. Two hours after the stimulation, the production of inflammatory mediators (PGE_2 and LTB_4) and western blots analysis (leptin receptor) were performed. Western blot assay showed that expression of long-form leptin receptor is decreased (63%) in the primary cultures of endothelial cells derived from intrauterine malnourished rats. Leptin alone did not induce any alteration on the levels of the inflammatory mediator evaluated, whereas LPS increased the PGE_2 (250%) and LTB_4 (29%) levels. Only in endothelial cells from nourished rats, leptin enhanced lipid mediators production induced by LPS (PGE_2 - 28% and LTB_4 - 18%). Interestingly, the same was not observed in endothelial cells from intrauterine malnourished rats. **Conclusion**: Our preliminary results suggest that intrauterine malnutrition dowregulates leptin receptor expression and modulate lipid mediators production in primary culture of pulmonary endothelial cells stimulated by LPS. **Financial support**: FAPESP (2010/01404-0, 2012/51104-8) and CNPq. **Animal Research Ethical Committee**: CEP 1666/99.

04.020 Involvement of 11-bHSD-1/2 in altered inflammatory response pattern presented by undernourished offspring. Vaz DBR[1], Balbino AM[1], Akamine EH[2], Carvalho MHC[2], Landgraf RG[1], Landgraf MA[2,1] [1]Unifesp-Diadema – Inflamação e Farmacologia Vascular, [2]USP – Farmacologia

Introduction: Intrauterine malnutrition can result in impairment of inflammatory response; action of glucocorticoid on its receptors can be involved in these alterations. Here, we have investigated the impact of local regulation of glucocorticoids on the development of acute lung injury, in intrauterine undernourished rats, at 12-week-of age. **Materials and Methods**: Intrauterine undernourished male offspring were obtained from dams that were fed 50% of the nourished diet of counterparts. At 12-week-of age, the response to LPS intratracheal instillation *(*750□g/200□L) was evaluated*; we* also measured IL-6, IL-1□, TNF-□ and IFN-□ levels (by Multiplex), and GR (glucocorticoid receptor), 11-□HSD-1 and 11-□HSD-2 mRNA levels (by real time PCR), in lung tissue. **Results**: Intrauterine undernourishment reduced cell infiltration into airways after LPS instillation, but increased TNF-α (132%) and IFN-□ (180%) expression, compared to nourished offspring. No difference was observed in IL-1□ and IL-6 levels, when compared both stimulated groups, and in GR and 11-□HSD-1 mRNA levels, when compare both non stimulated groups. After LPS instillation, different from undernourished offspring, nourished offspring presented reduction in GR and 11-□HSD-1 mRNA levels. Under basal conditions, undernourished offspring showed lower levels of mRNA for 11-□HSD-2, compared to nourished offspring (54%); LPS instillation induced significant reduction in 11-□HSD-2 mRNA levels in both groups (80% in nourished and 23% in undernourished group). **Conclusion**: Our preliminary results indicate that imbalance in cytokines expression and local regulation of glucocorticoids action may be involved in altered inflammatory response pattern presented by undernourished offspring. **Supported by** FAPESP-2012/51104-8, 2010/01404-0, CNPq-447303/2014-7 Animal Research Ethical Committee: (CEUA–67/2013)

04.021 TRPC5 regulates temperature and body weight in septic mice. Pereira DMS[1], Mendes SJF[1], Castro Jr JAA[1], Aubdool A[2], Alawi K[2], Takore P[2], Grisotto MAG[1], Brain S[2], Fernandes ES[3] – [1]Universidade Ceuma – Biologia Parasitária, [2]King's College London – Vascular Biology and Inflammation, Cardiovascular Division, [3]Universidade Ceuma and King´s College London

Introduction: Transient receptor potential (TRP) channels are non-selective cation channels expressed on neuronal and non-neuronal cells, known to be involved in several pathophysiological processes in the organism. Recently, a protective role for the TRP vanilloid 1 channel was suggested in sepsis, and this was associated with its ability in modulating the immune response, especially in respect to oxidative stress. However, little is known of the role other TRP channels may play in this syndrome. TRP cannonical 5 (TRPC5) is predominantly expressed on neuronal cells and it can be activated by a range of endogenous molecules including nitric oxide, hydrogen peroxide and reduced thioredoxin. **Aims**: Here, we investigated for the first time, the involvement of TRPC5 in sepsis. **Methods**: For this, sepsis was induced by an intraperitoneal injection of lypolissacharide (LPS;E. coli, serotype 0111: B4,11.25 millions of EU/kg) in wild type (WT) and TRPC5 knockout (TRPC5KO) mice (2-months old). Vehicle-treated animals were used as controls (PBS;10 ml/kg). Systemic parameters such as body weight and temperature were evaluated, by comparing baseline readings with those obtained 24h following sepsis induction. Blood pressure was measured by tail-cuff plethysmography at baseline and 24h after sepsis, following a 15-day training period (15-cycle readings of 30 seconds each per day). The severity of sepsis was evaluated at 24h following LPS injection. For this, a score was attributed to each of the following parameters: grooming behaviour (1- normal grooming, 2- reduced grooming, 3- no grooming), mobility (1-normal mobility, 2-partial impairment, 3-poor mobility, 4-no mobility), piloerection (1- absence, 2- presence) and weeping eyes (1- absence, 2- presence). The summation of the scores attributed to each of the parameters for each animal was taken as severity score index, with the highest scores corresponding to the worst outcome of disease. For comparison, baseline scores were taken for all groups of mice. **Results**: Sepsis evoked loss of body weight in addition to a marked drop in blood pressure in WT mice in comparison with vehicle-treated controls ($p<0.05$). These changes were associated with disease severity, characterized by reduced grooming and mobility, presence of piloerection and weeping eyes. TRPC5KO mice exhibited similar blood pressure readings (both at baseline and 24h after sepsis induction) to those of WT mice. Also, sepsis severity in TRPC5KO mice was similar to that of WT animals. On the other hand, TRPC5KO mice presented with increased hypothermia in comparison with their vehicle-treated counterparts and also in comparison with LPS-treated WT animals ($p<0.05$). No significant changes in body weight were observed in LPS-injected TRPC5KO when compared to vehicle-treated TRPC5KO mice. Conclusions: Although TRPC5 deletion does not seem to interfere with sepsis severity at 24h post LPS injection, TRPC5 seems to mediate temperature regulation and body weight in septic mice. However, more experiments are necessary to further understand the contribution of this channel to this syndrome. **Financial support**: FAPEMA, CNPq and CAPES Research was conducted in accordance with the UK HO Animals (Scientific Procedures) Act of 1986 and local King’s College London ethics approval (HOLC I417F8A77).

04.022 Immunomodulatory properties of Braylin from *Z. tingoassuiba* Espírito Santo RF[1], Meira CS[2], Costa RS[3], Souza Filho OP[3], Velozo ES[3], Soares MBP[2], Villarreal CF[1] [1]UFBA – Farmacologia e Terapêutica Experimental, [2]CPqGM-LETI-Fiocruz-BA, [3]UFBA – Pesquisa em Matéria Médica

Introduction: Natural products have been a source of compounds with immunomodulatory activities. Coumarins are compounds known for their pharmacological potential as anti-inflammatory, antioxidant, antiviral, antimicrobial and antitumor (Shokoohinia, Y, Adv Pharmacol Sci, 847574, 1, 2014). Braylin is a coumarin found in H. oreadica, H. brasiliana and H. superba (Severino, VGP, Molecules, 19, 12031, 2014). To date, few studies have investigated the pharmacological properties of braylin. **Objective**: This study aimed to evaluate the immunomodulatory properties of braylin in experimental models. **Methods**: Braylin was isolated from the roots of Z. tingoassuiba (Rutacea) collected in August 2009 in Jaiba district, locatized in the city of Feira de Santana, in Bahia State, Brazil and received botanic identification by Herbarium Alexandre Leal Costa (ALCB), Federal University of Bahia, Brazil.• @Initially, the cytotoxicity of braylin was determined in J774 macrophage lineage. Immunomodulatory activity of braylin was next evaluated in activation-induced macrophage cytokine and nitric oxide (NO) production and in a lymphoproliferation assay with spleen cells obtained from Swiss mice (male, 20-25 g). The proliferation rate of lymphocytes challenged with concavalin A was assessed by 3H-thymidine incorporation. Cytokines (IL-6, IL-10, IL-1β and TNF-α) concentrations in supernatants from macrophage cultures were determined by enzyme-linked immunosorbent assay (ELISA). Nitric oxide production was estimated by measuring nitrite content in supernatants using the Griess method. **Results**: Addition of braylin (10 – 40 μM) to macrophage cultures stimulated with lipopolysaccaride (LPS) and interferon-γ (INF-γ) caused a concentration-dependent inhibition in NO production, as indicated by the nitrite concentrations in supernatants ($P < 0.05$). The effects of braylin on NO production by activated macrophages were not due to toxicity, as J774 macrophage cultured in the presence of braylin at 40 μM showed no cytotoxicity. The effects of braylin on cytokine production by activated macrophages were next evaluated. Addition of braylin (10 – 40 μM) to macrophage cultures stimulated with LPS and INF-γ significantly ($P < 0.05$) decreased the TNF-α, IL-1β and IL-6 production. This effect was concentration-dependent. The release of IL-10 by macrophages was not modulated by braylin. Addition of braylin (5 – 40 μM) in cultures of lymphocytes stimulated with Con A caused a concentration-dependent inhibition of proliferation ($P < 0.05$). Braylin at 40μM caused an inhibition of proliferation of 97%. In contrast, dexamethasone caused an inhibition of lymphocytes proliferation of 73%. **Conclusion**: The present study demonstrated for the first time that braylin has potent immunosuppressive activities in macrophages and lymphocytes cultures. The effect of braylin was greater than of dexamethasone, the gold standard of glucocorticoids, suggesting that this coumarin may be useful for the development of new immunosuppressive drugs. **Financial Support**: CNPQ, FAPESB Animal Research Ethical Committee: L-IGM-025/09

04.023 Role of endothelin receptor antagonists in primary culture of lung endothelial cells activated by LPS. Silva MM[1], Balbino AM[1], Gil NL[1], Azevedo GA[1], Fernandes L[1], Landgraf MA[2,1], Landgraf RG[1] [1]Unifesp-Diadema – Laboratório de Inflamação e Farmacologia Vascular, [2]USP – Farmacologia

Introduction: The levels of endothelins in the lung tissue are particularly high compared to other tissues. Within the lung endothelins are synthesized by the epithelial cells of the airway, endothelial cells and inflammatory cells. Vascular endothelium is closely related with the circulatory control, and has an important participation in cellular and molecular events which occurred during immune system reactions and tissue injuries. **Objectives**: Characterization of primary culture of lung endothelial cells and evaluation the role of endothelin receptor in rat pulmonary endothelial cells and its role in the production of prostaglandin E_2 and cytokines. **Methods**: Male Wistar rats were euthanized with overdose of ketamine and xylazine and lung tissue samples were isolated under sterile conditions. These cells were characterized by immunofluorescence using ULEX and von Willebrand factor, which is a traditional marker of endothelial cells, and also by flow cytometry using antibodies CD54(ICAM-1), CD105(endoglin), CD106(V-CAM) and CD45(hematopoietic cells). After the characterization, these cells were stimulated or not with LPS(1µg/mL), endothelin A receptor antagonist (BQ123, 10ng/mL) or endothelin B receptor antagonist (BQ788, 10ng/mL), for 6h to evaluate the prostaglandin E2 (EIA) and cytokines (multiplex) production. **Results**: Cells were positive for all markers used, except for CD45. Endothelin receptor antagonist (ETA or ETB) alone did not induce any alteration on the levels of the inflammatory mediator evaluated or cytokine production, whereas LPS increased the PGE_2 (119%) levels and reduced (80%) after BQ788 administration. LPS increased levels of IL1-β, IL-2, TNF-α and GMCSF. This increase was reduced after administration of endothelin receptor antagonists (ETA or ETB). Only ETB receptor treatment decreases the IL-1β production. **Conclusion**: Our preliminary results suggest that endothelins plays an important pro-inflammatory role in the cultures of rat primary endothelial cells stimulated by LPS. **Financial support**: FAPESP (2010/01404-0, 2012/51104-8) and CNPq. Animal Research Ethical Committee: CEP 1666/99.

04.024 Role of leptin receptor and TLR-4 in reduced acute lung inflammation, in intrauterine undernourished mice model. Balbino AM[1], Fernandes L[1], Landgraf MA[1,2], Landgraf RG[1] [1]Unifesp-Diadema – Inflamação e Farmacologia Vascular, [2]USP – Farmacologia

Introduction: Lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, plays an important role in the development of acute lung injury (ALI) and exerts its biological effects on the host by binding to Toll-like receptor 4 (TLR4). Intrauterine undernourishment is associated with decreased lung function, in adulthood, and reduced lung allergic inflammation. **Objectives**: In this study we standardized a model of intrauterine undernourishment in mice and we also have investigated the influence of intrauterine undernourishment on the TLR-4 expression. **Methods**: Female C57Bl/6 mice were randomly divided into 2 groups: nourished (ad libitum diet) and undernourished (30% food restriction). After birth, each litter was left with the mother for 28 days. 5-6 male C57BL/6 mice at 8-9 wk of age were used for each group. Control group was given saline intranasally (i.n., *30µL).* Experimental groups were given LPS (i.n., 1.5µg/g/30µL)*.* 6h after instillation, the bronchoalveolar lavage fluid (BALF) was collected to evaluate cellular infiltration in lung. Lungs were removed for measurement of the cytokines and chemokines production (multiplex) and TLR-4 expression and long-form leptin receptor by western blot. **Results**: Intrauterine undernourished mice (UR) stimulated by LPS were presented significantly reduced in total cell (45.9%) and neutrophils (76.2%) in bronchoalveolar lavage fluid when compared to nourished group (NR). Besides, UR mice also exhibited an reduced levels of cytokine expression profile (TNF-α, IL-1β, KC and GCSF expression) than NR mice following LPS administration. Western blot assay showed that expression of long-form leptin receptor and TLR-4 is decreased (39.8% and 45%, respectively) in UR group when compared to NR group. **Conclusion**: Our preliminary results suggest that intrauterine undernourishment downregulates leptin receptor and TLR-4 expression and decreased levels of proinflammatory cytokines modulate acute lung inflammatory response induced by LPS. **Financial support**: FAPESP (2010/01404-0, 2012/51104-8) and CNPq. Animal Research Ethical Committee: CEP 1666/99.

04.025 Tumoral necrosis factor-alpha inhibits the increase of cytosolic calcium levels and C-SRC and fibrinogen receptor activation in ADP-stimulated platelets.

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Introduction: Platelets have been described as important cells in inflammation; however, the effects of cytokines on platelet reactivity are rarely studied. Tumor necrosis factor alpha (TNF-α) is an essential mediator in the pathogenesis of many inflammatory and cardiovascular disorders. Therefore, the objective of the present work was to investigate the effects of TNF-α in platelets. **Methods**: Blood from abdominal aorta of male Wistar rats (250-320g) was collected in ACD-C (9: 1 v/v). Platelet-rich plasma (PRP) was obtained by centrifugation of whole blood at 200 g for 15 min. The platelets were washed using citrated buffer (pH 6.0) and the number was adjusted to $1.2x10^8$ plat/ml. Platelet aggregation was measured in a two channel aggregometer (Chronolog Lumi-Aggregometer). Aggregation assays were carried out incubating platelets with increasing TNF-α concentrations (1 – 3000 pg/ml) for different intervals of times (5 – 60 min) before addition of ADP or thrombin. Some experiments were carried out in the presence of the non-selective antagonist of TNFR1 and TNFR2, R7050. Effect of TNF-α on platelet viability was determined using MTT. The effect of TNF-α on the Ca^{++} mobilization in platelets was investigated through fluorescence assays using fluo-3AM and Western blotting assays were carried out to determine the activation of c-Src and the fibrinogen receptor. Finally, the cAMP and cGMP levels in platelets were determined by ELISA. **Results**: TNF-α dose- and time-dependently inhibited ADP or thrombin-induced platelet aggregation. The inhibitory effect of TNF-α on ADP(5 µM)-induced platelet aggregation was maximum in a concentration of 300 pg/ml incubated with platelets for 30 min (90 ± 7% of inhibition), which was significantly prevented by R7050. Platelet viability was not modified by TNF-α (30 and 3000 pg/ml) incubated for 5 to 60 min. Incubation of platelets with TNF-α (300 pg/ml, 30 min) reduced the increased total Ca^{++} concentration induced by thrombin (200 mU/ml) by 53%. TNF-α decreased Ca^{++} internal mobilization (1,8 fold) and external Ca^{++} influx (3,4 fold) compared to thrombin-stimulated platelets in absence of this interleukin. TNF-α reduced the cAMP levels in ADP-activated platelets by 60%. On the other hand, TNF-α significantly increased cGMP levels in ADP-activated platelets (51% increase). Pre-incubation of platelets with the guanylyl cyclase inhibitor ODQ did not modify the inhibitory effect of TNF-α on ADP-induced platelet aggregation. Western blotting analysis showed that TNF-α significantly reduced phosphorylation on Tyr416 of c-Src and 37% the Tyr773 phosphorylation of β3 subunit of αIIbβ3 integrin (fibrinogen receptor) in ADP-activated platelets. **Conclusion**: Our results show that TNF-α inhibits platelet aggregation via TNFR1 and/or TNFR2 receptors, without affecting platelet viability. The inhibitory effect of TNF-α on aggregation is accompanied by a reduction in cytosolic Ca^{++} and the inhibition of c-Src and fibrinogen receptor activation, which are cAMP and cGMP-independent effects. **Supported**: CAPES **Committee for Ethics in Animal Research** (State University of Campinas – UNICAMP) protocol number: 2947-1

04.026 Role of atypical chemokine receptor ACKR2 (D6) in the lung inflammatory response caused by silica particles in mice. Pereira JG[1], Dias DF[1], Ferreira TPT[1], Azevedo RB[1], Teixeira MM[2], Graham G[3], Martins MA[1], Silva PMR[1] [1]Fiocruz – Fisiologia e Farmacodinâmica, [2]UFMG – Farmacologia, [3]University of Glasgow – Infection, Immunity and Inflammation,

Introduction: Silicosis is a chronic occupational disease caused by inhalation of free crystalline silica particles and is characterized by intense inflammation and fibrotic response. Chemokines act in controlling cell recruitment and function via GPCR receptors expressed on some cell populations. ACKR2 is a member of atypical receptors which bind chemokines with high affinity and specificity, but appears incapable to couple signaling pathways. As the role of ACKR2 in chronic diseases is not sufficiently clarified, this study tested whether ACKR2 regulates inflammation and fibrosis during silica-induced lung disease in mice. **Aims**: As the role of ACKR2 in chronic diseases is not sufficiently clarified, this study tested whether ACKR2 regulates inflammation and fibrosis during silica-induced lung disease in mice. **Methods**: ACKR2 knockout (KO) and wild type littermate (C57/Black 6 - WT) mice were instilled intranasally with silica particles (10 mg; 0.5 - 10 µm) and with saline (controls). Analyzes were made at different time points, varying from 1 - 28 days after silica provocation, and included: i) body weight (g), ii) lung function and airways hyper-reactivity to cholinergic agonist methacholine (invasive plethysmography, Finepointe Buxco System) and iii) morphological and morphometric analyses of lung tissue (Hematoxylin & Eosin and Picrus sirius staining). All experimental procedures were approved by the Committee on Use of Laboratory Animals of Oswaldo Cruz Foundation (license LW 57/14). **Results**: We noted that both groups WT and ACKR2 KO mice showed loss of body weight on days 1 and 3 after silica challenge, when compared to controls. No difference was noted until 28 days post-silica. Unexpectedly, reduced lung leukocyte infiltration, fibrosis and granuloma formation were noted in ACKR2 KO silicotic in comparison to WT mice. Also, a significant attenuation of basal lung function alterations (increased resistance and elastance) as well as airways hyper-reactivity to aerosolized methacholine was noted following silica provocation in the knockout mice. **Conclusion**: Our findings reveal that silica-challenged ACKR2 knockout mice had reduced fibrosis, lung function alteration and airways responses to methacholine when compared to WT animals. In addition, we can propose that a novel function for ACKR2 may be considered under conditions of tissue fibrosis. Additional studies are needed to more fully characterize the involvement of ACKR2 in chronic lung fibrotic diseases such as silicosis. **Financial support**: FIOCRUZ, CNPq, FAPERJ, CAPES and European Community (UE FP7- 2007-2013 - n°HEALTH-F4-2011-281608).

04.027 Gabapentin reduces pro-inflammatory parameters of the colitis induced by Trinitrobenzenesulfonic Acid (TNBS) in rats. Magalhães DA[1], Cruz Junior JS[2], Dutra YM[2], Brito TV[1], Filgueiras MC[2], Barbosa ALR[2] [1]UFPI – Biotecnologia, [2]UFPI

Introduction: Gabapentin (GBP) is used in anticonvulsant clinical behavior. Recently data were demonstrated that GBP reduced the inflammatory response in systemic inflammation models (Dias, 2014). However, there are no studies that have shown their anti-inflammatory action in the gut. Objective: To evaluate the activity of GBP on the macroscopic scores, wet colon weight and the concentration of MPO in the trinitrobenzenesulfonic acid induced colitis in rats. **Methods**: In the experiments involving TNBS-induced colitis, rats were treated with GBP (0.6, 3 and 15 mg/kg; orally, p.o.) or dexamethasone (Dexa: 1 mg/kg; subcutaneously, s.c.) once daily for 3 days before and after TNBS instillation (only on first day). On the third day after the TNBS-colitis induction, the rats were sacrificed by cervical dislocation for analysis of inflammatory parameters using 5 cm of the distal colon tissue region, were evaluation of macroscopic scores of lesion was performed by modifying the criteria previously described Morris (1989) and were weighed to determinate the colon oedema. The results were expressed in increase in colon weight (g)/5 cm ratios, compared with a normal control group, without colitis. Additionally, samples of intestinal tissue were then removed for the measurement of concentration myeloperoxidase (MPO) in the colon of rats. The study was approved by Ethics Committee of the Federal University of Piauí (protocol n° 011/15). **Results**: The administration intracolon of TNBS induced a significant increase of colon macroscopic intestinal lesions (22.20 ± 0.58 scores of lesion) when compared with GBP reduced (0.6 mg/kg: 20.40 ± 2.60 scores of lesion; 3 mg/kg: 18.60 ± 1.47 scores of lesion and 15 mg/kg: 12.75 ± 2.25 scores of lesion) the damage scores of macroscopic lesions in the colon tissue, with the maximal effect observed at a dose of 15 mg/kg. Similar effects were produced by Dexa (14.40 ± 2.40 scores of lesion). The TNBS induced a significant increase in the colon weight (1.13 ± 0.04 g) as compared with Dexa Group (0.90 ± 0.05). However, pretreatment with GBP reduced (0.6 mg/kg: 1.20 ± 0.05 g; 3 mg/kg: 1.04 ± 0.09 g and 15 mg/kg: 0.90 ± 0.08 g) the colon injury after TNBS colonic instillation. The TNBS into the colon determined MPO activity in the concentration of 152.7 ± 4.65 units of MPO/mg of colon tissue, while the group treated with GBP 15 mg/kg decreased an activity of this enzyme at 65.70 ± 17.70 UMPO/mg of colon tissue. **Conclusion**: This study showed that GBP has a protector effect on the TNBS-induced colitis in rats by the anti-inflammatory effect in macroscopic scores and wet weight of the colon, as well as mechanisms involving inhibition of infiltration of inflammatory cells into the colon. Thus, we suggest that GBP may have potential applications in the development new therapeutic targets against inflammatory bowel disease in humans. **Financial support**: CNPq/FAPEPI
DIAS, J.M. Gabapentin, a Synthetic Analogue of Gamma Aminobutyric Acid, Reverses Systemic Acute Inflammation and Oxidative Stress in Mice. Inflammation v.37; p. 1826-1836, 2014; MORRIS, G.P. Hapten-induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterology* v. 96; p.795, 1989.

04.028 Reduction of mast cell number and reactivity induced by glucocorticoids is associated with up-regulation of advanced glycation end-products receptors expression. Santoro T[1], Torres RC[1,2], Insuella DBR[1], Martins MA[1], Silva PMR[1], Carvalho VF[1] – [1]Fiocruz, [2]UFRJ

Introduction: Glucocorticoids (GC) are anti-inflammatory drugs used in clinic for treatment of chronic inflammatory diseases. Some of the anti-inflammatory effects of GC therapy are inhibition of generation of pro-inflammatory mediators and reduction in the number of several inflammatory cells, such as mast cells (MC). In addition of anti-inflammatory actions of GC, its present several metabolic effects, including hyperglycaemia with concomitant generation of advanced glication end-products (AGEs) and muscle atrophy. AGEs, like glycated albumin, induces apoptosis of mast cells through activation of its receptors RAGE and galectin-3. In this study, we take to investigate the role of AGEs in reducing MC number and reactivity induced by GC. **Methodology**: Male Wistar rats were received GC, dexamethasone (DX) or prednisolone (PD) (0.1 mg/kg; sc.) once a day, during 21 days. Some rats were treated with aminoguanidine (AG) (50 or 250 mg/kg; v.o.) or L-NAME (30 mg/kg; v.o.) daily, started on the day 4 after first administration of GC. 24h after the last administration of the drugs, we analyzed number of MC through toluidine blue staining; MC reactivity by means of histamine quantification after immunologic (passive reaction) or non-immunological (48/80 compound) stimulation; blood glucose and weight gain; plasma fructosamine through biochemical kit; muscle atrophy by analysis of histological slices; expression of AGEs receptors, RAGE and Galectin-3, by western blot. **Results and Discussion**: Treatment with DX or PD decreased number and reactivity of MC compared to untreated rats. Concomitant treatment with AG (250 mg/kg), which induces AGEs scavenger, was able to restore the reduction as well in number as in reactivity of mast cells induced by either DX or PD. However, treatment of rats with AG (50 mg/kg), dose which does not induces AGEs scavenger but only inhibit NOS activity, or L-NAME, non-selective NOS inhibitor, does not interfere with the effect of DX on number and reactivity of mast cells. Thus, we can suppose that the reduction of the number and reactivity of MC induced by GC is somehow related to AGEs. By analyzing the blood, we observed that neither DX nor PD were able to change both glycaemia and fructosamine (glycated albumin). So, as the GCs did not interfere with AGEs formation, we analyzed the expression of RAGEs in mast cells. We observed that treatment with DX increases the expression of both RAGE and galectin-3, and the treatment with AG (250 mg/kg) restores the expression of these receptors to basal levels. Finally, we evaluated some metabolic effects of GC treatment. We observed a characteristic reduction in weight gain and muscle atrophy in DX treated group, and these parameters were not changed by the treatment together with AG (250 mg/kg). Thus, we can conclude that the mechanism by which GC reduces the number and reactivity of MC is associated with overexpression of AGEs receptors. Furthermore, for the first time we dissociated an anti-inflammatory activity of the metabolic effect of glucocorticoids. **Financial Support**: FAPERJ, CNPq and FIOCRUZ. **Animal Research Ethical Committee Approval**: Ethic Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation, License LW – 23/11.

04.029 Evaluation of anti-inflammatory potential of hydroalcoholic extract and polysaccharide fraction from *Thuja occidentalis* in mice. Silva IS, Brito CFC, Sousa FBM, Carvalho NS, Araújo S, Souza LKM, Araújo TSL, Pacífico DM, Filho ACML, Lima GM, Almendra RB, Medeiros JVR UFPI – Farmacologia

Introduction: *Thuja occidentalis*, a tree belonging to the family Cupressaceae, has demonstrated pharmacological properties. Inflammation is a well-coordinated series of events that depends on increased vascular permeability and sequential release of inflammatory mediators. The most commonly drugs used to treat inflammatory conditions are associated with low therapeutic efficacy and adverse effects. **Aims**: This study investigated the anti-inflammatory effect of hydroalcoholic extract and the polysaccharide fraction from *Thuja occidentalis* in mice and its possible mechanisms. **Methods**: The present study was approved by the local ethics committee (Protocol no. 0066/10). The substances were used in experimental models of inflammation (Paw Edema induced by different agents, Carrageenan-induced Peritonitis and Glutathione levels). **Results**: The carrageenan injected in the right hind paw promoted the formation of 3h edema (0.128 ± 0.005 ml). The pretreatment with hydroalcoholic extract (HAE) and the polysaccharide fraction (PLF) from *Thuja occidentalis* (3, 10, and 30 mg/kg) significantly reduced the edematogenic response, and the maximum effect was observed after a dose of 3 mg/kg, which significantly ($p^*<0.05$) reduced the 3h edema (0: 05 ± 0.005 ml; 60.9% reduction and 0: 03 ± 0.007 ml; 76.5% decrease, respectively). In the paw edema test induced by different agents, the pretreatment with HAE and PLF (3 mg/kg) inhibited significantly ($p^*<0.05$) paw edema induced by compound 48/80 (0024 ± 0.005 ml; 61.2% inhibition and 0: 03 ± 0.003 ml; 53.8% inhibition), bradykinin (0.002 ± 0.002 mL; 87.5% inhibition and 0.006 ± 0.002 ml; 64% inhibition), dextran (0.016 ± 0.003 mL, 63.6% inhibition and 12: 01 ± 0.004 mL; 77.27% inhibition), PGE2 (12: 04 ± 0.007 mL; 55.5% inhibition and 0.026 ± 0.011 mL; 71.11% inhibition), histamine (0.010 ± 0.002 mL; 83.3% inhibition and 0.016 ± 0.004 ml; 76.11% inhibition) and serotonin (0.01 ± 0.005 ml; 80% inhibition and 0.018 ± 0.002 ml; 66.6 % inhibition), respectively. In carrageenan-induced peritonitis test (500µg/cavity), cell migration was found after 4 hours of its administration, with a total leukocyte count (WBC) (8.4 ± 0: 28 $\times 10^6$ cells/mL) and neutrophils (NC) (6, 06 ± 0. 6193 $x10^6$ neutrophils/mL). The pretreatment with HAE and the PLF (3.0 mg/kg, ip) reduced the WBC in the peritoneum (4.0 ± 0.5 and 5,22 ± 0.61 $x10^6$ leukocytes/ml respectively) and reduced the NC migration (2.93 ± 0.17 and 6,06 ± 0,6193 $\times 10^6$ neutrophils/ml). Treatment with carrageenan (500 ug/cavity) resulted in decreased concentrations of GSH (69.74 ± 23.00) in mice compared to the control group treated with saline ($p^*>0.05$). Pretreatment with HAE and the PLF significantly increased the levels of GSH (116,8 ± 1,862 and 127,1 ± 2,191 respectively). **Conclusion**: The HAE and the PLC from *Thuja occidentalis* reduce the inflammatory response in mice by inhibiting the release of inflammatory mediators, cell migration and increased GSH levels. **Financial support**: CNPQ and FAPEPI

04.030 Anti-inflammatory activity of low power laser in classic experimental model of paw oedema acute in mice. Batista JA, Brito TV, Queiroz FFSN, Lima Filho ACM, Almendra RB, Macêdo WBS, Costa MS, Barbosa ALR, Filgueiras MC UFPI – Farmacologia

Introduction: Edema is one of the classic signs of acute inflammation and it's characterized by excessive fluid volume in the interstitial tissue formed by the increase of microcirculation permeability. Treatment of the inflammatory conditions is performed in a traditional manner by drugs such as dexamethasone, diclofenac and ibuprofen. In addition, alternative resources has taken attention in the clinical area such as laser therapy, where the advantages and the various effects caused by such treatment has already been well explained in the literature, including the inflammatory modulation and edema reduction. **Aims**: We aimed to investigate the effect of therapy in a dose laser manner, in acute inflammatory processes in the classical model of paw edema in rats. **Methods**: The edema was induced by injection into the right hind paw of the mouse of 50µL suspension of carrageenan (Cg, 500µg / paw). The measurement of edema volume was given 1 hour after carrageenan application. The application of the low intensity laser (InGaAs), 904 nm wavelength, occurred during those intervals mentioned at dose of 1 J/cm². Measurement was made over a period of 6 to 96 hours to observe the effect of laser therapy in acute and chronic conditions, respectively. After 6 hours of the carrageenan administration, half of the animals were sacrificed and excised the plantar tissue for quantification of neutrophil migration, wich was performed through measurement of myeloperoxidase (MPO). **Results**: It was demonstrated that the group treated with the therapeutic laser dosimetry of 1J/cm^2 showed a decrease in volume over a 6 hour period (0.048 ± 0.01 U / mg of tissue) as compared with carrageenan group (59, 33 11 ± 55 U / mg of tissue) and the dosimetry of 1 J/cm^2 caused a decrease in paw volume of the animals after the first 24 hours (0.073 ± 0.016 U / mg of tissue). It was seen in the group treated with the density of 1 J/cm^2 decreased the enzyme migration to 43.86 ± 9.07 U/mg tissue. **Conclusion**: It was found that the laser has anti-inflammatory activity at the doses tested, reducing paw edema and neutrophils migration to the site of injury. **Ethics Committee Number**: (Faculdade Integral Diferencial—FACID, number of protocol: 002/13.) **Support**: CNPQ and FAPEPI.

04.031 Does hydrogen sulfide (H_2S) influence apoptosis process in lungs from allergic mice? Ribeiro MC[1], Mendes JA[2], Silva MS[1], Moreira GCP[1], Dias NH[1], Albaladejo BT[1], Pereira JA[1], Rocha T[1], Ferreira HHA[3] – [1]USF, [2]Unicamp, [3]SLMandic

Introduction: Many studies have shown that hydrogen sulfide (H_2S) has a relevant role in the pathophysiology of lung diseases. **Aim**: Investigate the effect of H_2S in modulating apoptosis in lungs from allergic mice. **Methods**: BALB-C mice were sensitized subcutaneous with 100 mg of ovalbumin (OVA) and challenged intranasally with 10 µg of OVA, twice daily (OVA group). Some sensitized mice received sterile saline without OVA at the time of challenge (saline group). Others mice were sensitized but received treatment with H_2S donor - Sodium hydrosulfide (NaHS; 14 µmol/Kg) 30 minutes before each OVA-challenge (OVA/NaHS group). The euthanasia was performed 48 hours after allergen challenge. Bronchoalveolar lavage (BAL) was collected for eosinophils isolation by immunomagnetic method. The right lung was, then, removed, and homogenized to study the expression of caspase 3, caspase 9, Bax and Fas-L. The left lobe was fixed in formalin for 1) histological analysis of lung parenchyma inflammatory cell infiltrate using hematoxylin/eosin staining (HE); 2) the *in situ* apoptosis by TUNEL assay and 3) verification of expression of cistationia-ɣ-lyase enzymes (CSE) and cystathionine-β-synthase (CBS) by immunohistochemistry. **Results**: The histological results showed an inflammatory infiltrate around the bronchi and bronchioles in the OVA group, with a prevalence of eosinophils, which was prevented by NaHS-treatment (64% reduction). The treatment of allergic mice with NaHS also decreased the expression of caspase 3 [Caspase 3/□ actin (relative density): 0.87 ± 0.03 and 0.67 ± 0.08 for OVA and NaHS-treated group, respectively] and Fas-L (relative density: 1.34 ± 0.06 and 0.94 ± 0.02 for OVA group and NaHS-treated mice, respectively). Bax and caspase 9 expressions were not modified. OVA-challenge or NaHS-treatment was unable to modulate the apoptosis of BAL eosinophils (0,55% ± 0.15 approximately). However, the NaHS avoid the apoptosis increase in bronchial epithelial cells promoted by OVA challenge (OVA = 2.5 ± 0.05 *versus* NaHS-treated group = 1.7 ± 0.01 apoptotic cells/mm^2). Forty percent increase in the expression of CSE enzyme in the bronchial epithelium and 120% in the vascular endothelium were observed in the lungs of allergic mice as compared to saline mice, that were amplified by NaHS-treatment (72% and 37% for OVA and NaHS-treated group). CBS expression showed increase of 40% and 370% in bronchial epithelium and vascular endothelium, respectively, but NaHS-treatment did not induce any modification. **Conclusions**: Our results suggest that NaHS-treatment prevented apoptosis and, consequently, the bronchial epithelium destruction, which contributes to the pulmonary inflammation decrease. The CSE enzyme may be involved in this process. Therefore, the H_2S can have a protective effect against lung damage caused by allergic reaction, representing a potential therapeutic agent for allergic pulmonary disorders, such as asthma. **Financial Support**: Fapesp and CNPq; Animal Research Ethical Committee San Francisco University, Brazil; process number 001.03.12.

04.032 L-amino acid oxidase from *Bothrops jararaca* **snake venom increases vascular permeability in rat dorsal skin**: **involvement of free radicals.** Fonseca FV, Marcelino EP, Pereira BB, Panunto PC, Torres Huaco FD, da Silva RF, Hyslop S FCM-Unicamp – Biochemical Pharmacology

Introduction: Snake venom L-amino acid oxidases (LAAO) catalyze the oxidative deamination of stereospecific L-amino acids. Venom LAAOs have various biological activities but their role in venom-induced changes in permeability is unclear. In this work, we examined the ability of LAAO from *Bothrops jararaca* snake venom to alter vascular permeability in rats and assessed the involvement of free radicals. **Methods**: LAAO was purified by a combination of size-exclusion (Superdex 75) and ion-exchange (Q-Sepharose) chromatography; purity was assessed by SDS-PAGE and RP-HPLC. Changes in vascular permeability caused by venom and LAAO were assessed in the dorsal skin of male Wistar rats (300-350 g) anesthetized with sodium thiopental (50 mg/kg, i.p.). Plasma extravasation was measured based on the accumulation of human ^{125}I-serum albumin (2.5 □Ci/rat) injected i.v. along with Evans blue dye (25 mg/kg; marker dye). When required, superoxide dismutase (SOD; 1, 3, 10 and 30U/site), catalase (CAT; 1, 3, 10 and 30U/site) and N□-L-nitroarginine methyl ester (L-NAME, non-selective nitric oxide synthase inhibitor; 0.1, 1, 10 and 100mmol/site) were co-injected with venom or LAAO to assess the involvement of superoxide anion, hydrogen peroxide (H_2O_2) and nitric oxide, respectively, in the changes observed.The results were expressed as the volume (µl) of plasma accumulated at each skin site compared to total counts in 1 ml of plasma (determined in a □-counter). The experiments were approved by an institutional Committee for Ethics in Animal Use (CEUA/UNICAMP, protocol no. 2694-1). **Results**: Venom (0.1-100 µg/site) dose-dependently increased vascular permeability, with a maximal increase at ≥10 □g/site (extravasation of ~115□l vs. 6.8±1.0□l for Tyrode control).LAAO (0.25, 2.5, 7.5 and 25U/site) also significantly increased vascular permeability at the three highest doses (50.0±9.6, 74.1±8.1 and 84.6±9.0 □l, respectively; n=5; p<0.05vs.Tyrode control). SOD inhibited the LAAO (25 U/site)-induced increase in permeability by 44.5±1.5% and 41.9±3.8% with 3U/site and 30U/site, respectively; n=5; p<0.05 vs. Tyrode control); SOD also attenuated the venom (10 □g/site)-induced edema by 64.2±1.8% and 62.1±2.5% with 3U/site and 30U/site, respectively; n=5). In contrast to SOD, CAT (at all amounts tested) virtually abolished the LAAO-induced increase in permeability, with little dose-dependence(>90% inhibition for all doses; n=5);CAT inhibited venom-induced edema by >50%.As with CAT, L-NAME significantly attenuated the LAAO (25 U/site)-induced, but showed little dose-dependence (72.9±4.8%and 81.1±4.8% inhibition for 0.1 and 100 mmol/site, respectively; n=5); the venom-induced increase in permeability was also significantly inhibited by L-NAME (67.7±3.0% and 70.7±2.7% inhibition for 0.1 and 100 mmol/site, respectively; n=5). **Conclusion**: *Bothrops jararaca* venom LAAO increases vascular permeability in rat dorsal skin through mechanisms involving H_2O_2, nitric oxide and superoxide anion formation. This activity of LAAO may contribute to the local inflammatory response induced by *B.jararaca* venom in vivo. **Financial support**: CAPES, CNPq,FAPESP.

04.033 Comparative study of anti-inflammatory activity of *Mikania glomerata* and *Mikania laevigata* extracts. Pereira CS[1], Antunes E[1], Sawaya ACHF[2], Iwamoto RD[1], Landucci ECT[1] [1]FCM-Unicamp – Pharmacology, [2]IB-Unicamp – Plant Physiology

Introduction: The Mikania glomerata (MG) and Mikania laevigata (ML) are brazilian medicinal plants, popularly known as guaco, largely used for the treatment of various types of diseases that are the beneficial actions such as bronchodilator, anti-inflammatory, antispasmodic, in the treatment of gastric ulcers, among others. These species has been widely studied for having a similar composition and are commonly confused by similar leaf anatomy. Among the constituents present in guaco, coumarin is found in greater quantities and stands out for contributing to the anti-inflammatory effect. The widespread use, the minimal morphological differences and the large number of unsystematic information available in the literature justifies this project to clarify the differences between these two species, using coumarin as a positive control, through experimental models of inflammation such as paw edema and pleurisy. **Methods**: Male wistar rats (180 ± 220g) were anesthetized with isoflurane (2%) for paw edema and pleurisy procedures, respectively. The paw volume was measured immediately before the subplantar injection of 1 mg/paw of carrageenan (CG) and at selected time thereafter intervals, using a hydroplethysmometer. A skin incision was made to inject CG (1%) into the pleural cavity in a total volume of 0,1 mL. At 4 h thereafter, the animals were killed and the exudate and washings were removed by aspiration with PBS. Total leucocyte counts were performed in the pleural exudates. The rats were pretreated with saline (0,9%), MG and ML (200 mg/kg) and coumarin (75 mg/kg) by oral via, 1 h before injection of test agent. **Results**: The paw edema induced by CG, in pre-treated animals with guaco extract, was significantly inhibited by MG (0.4 ± 0.01 and 0.2 ± 0.02 mL/min, AUC for control and treated-rats, respectively; n=5; p<0.001), ML (0.4 ± 0.01 and 0.2 ± 0.01 mL/min, AUC for control and treated-rats, respectively; n=5; p<0,001) and coumarin (0.6 ± 0.02 and 0.4 ± 0.01 mL/min, AUC for control and treated-rats, respectively; n=5; p<0,001). In pleurisy, was observed a significant reduction in total leucocyte migration in response to ML (85±3 $x10^6$/cavity and 63±1 $x10^6$/cavity for control and treated-rats, respectively; n=5; p <0.001). On the other hand, MG had no significant effect (88±4 x 10^6/cavity and 79±3 $x10^6$/cavity for control and treated-rats, respectively; n=5). **Conclusion**: Our results demonstrate that *Mikania glomerata*, *Mikania laevigata* and coumarin promote significant anti-inflammatory effect on local edema. However, this same effect is not observed when it is a systemic edema, such as pleurisy. In pleurisy, only *Mikania laevigata* has proven effective on reducing of migration from total leucocytes. These experiments show that there are important differences in the anti-inflammatory action of these species, however, additional studies are needed to establish the effectiveness of this mechanism. **Financial Support**: CAPES. The present study was approved by the Committee for Ethics in Animal Research (State University of Campinas – UNICAMP, protocols number 3509-1 and 3863-1). **References**: CASTRO, R. C., Toxicon, vol. 38, pág. 1773, 2000. CZELUSNIAK, K.E., Rbpm, vol. 14, pág. 400, 2012.

04.034 Effect of systemic, spinal or local activation of a-Adrenoreceptors under the inflammatory process on the rheumatoid arthritis model induced by Zymosan. Alves HR[1], Lucena TO[1], Ferreira RT[1], Silva RF[1], Bassi GS[2], Vanderlinde FA[1], Kanashiro A[2], Malvar DC[1] [1]UFRRJ – Ciências Fisiológicas, [2]FMRP-USP – Farmacologia

Introduction: Rheumatoid arthritis (RA) is a chronic inflammatory disease that changes the synovial membranes of joints leading to destruction of bone and cartilage. Studies indicate that the sympathetic nervous system (SNS) may modulate the inflammation by activation of local β_2-adrenergic receptors, including RA. However, the role of local α-adrenergic receptors in the anti-inflammatory effect of SNS was not evaluated yet. Moreover, spinal α_2-adrenergic receptor has been reported in an anti-inflammatory neuroimmune pathway. **Aim**: To evaluate the effect of systemic, spinal or local administration of selective α-$_{1/2}$ adrenoreceptors agonists phenylephrine and clonidine, respectively, in the swelling and leukocyte migration on zymosan-induced arthritis. **Methods**: Male swiss mice (40-50g, n=6-10), were treated with saline (subcutaneous (s.c.), intra-tecal (i.t.) or intra-articular (i.a.)), phenylephrine or clonidine (0.01, 0.1, 1 mg/kg, s.c. or 0.01, 0.1, 1 µg, i.t. or i.a.). Zymosan (150 µg, i.a.) was injected 5 or 30 min before the drugs i.t. or s.c. injection, respectively, or co-administrated with the drugs i.a. injection. 6h after zymosan injection, knee joint swelling was evaluated by measurement of the transverse diameter of the knee joints using a caliper and the values were expressed as the difference (□) between the diameter measured before (basal) and in millimetres (mm). Immediately after, the determination of leukocyte (LK) migration was performed using a Neubauer chamber. **Results**: S.c. and i.a. injection of phenylephrine increased by 38.7%, 126.6% and 156.1% (s.c) and 48.3%, 98,3% and 176,3% (i.a.) the leukocyte migration on zymosan-induced arthritis when compared saline-treated group (1.73±0.18 and 1.18±0.12 $LKx10^4$, respectively). However, it was not observed reduction of knee joint swelling when compared with saline-treated group (0.80±0.19 and 1.49±0.12 mm, respectively). Moreover, i.t. injection of phenylephrine did not change the knee joint swelling nor leukocyte migration on zymosan-induced arthritis when compared saline-treated group. On the other hand, s.c., i.t. and i.a. injection of clonidine increased by 39.6%, 51.6% and 58.5% (s.c); 39.7%, 50,1% and 67,7% (i.t.) and 4.8%, 37,9% and 39.5% (i.a.) the leukocyte migration on zymosan-induced arthritis when compared saline-treated group (1.59±0.12; 1.56±0.12 and 1.24±0.05 $LKx10^4$, respectively). Moreover, i.t. or i.a. injection of clonidine inhibited by 10,4%, 34,4% and 33,8% (i.t.) and 24,4%, 48,3% and 33,3% (i.a.) the knee joint swelling induced by zymosan when compared with the saline-treated group (1,74±0.16 and 1.10±0.23 mm, respectively). Finally, s.c. injection of clonidine did not change the knee joint swelling induced by zymosan when compared with the saline-treated group. **Conclusions**: These results suggest that SNS produces pro- and anti-inflammatory effect on zymosan-induced arthritis through activation of local and/or systemic α_1- or α_2-adrenergic receptors, respectively. Moreover, activation of spinal α_2-adrenergic receptor produces anti-inflammatory effects on zymosan-induced arthritis. **Financial support**: CNPq. Ethical commission protocol nº 4096/2014/CEUA/IB/UFRRJ.

04.035 Human thioredoxin influences *Candida albicans* virulence *in vitro*. Silva BLR, Mendes SJF, Ferro TAF, Grisotto MAG, Monteiro Neto V, Fernandes ES
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Introduction: Thioredoxin (TRX) is a protein produced by all species, from bacteria to humans. Produced by the host during oxidative stress, TRX is a potent antioxidant, acting to downregulate an existing inflammatory response. However, host-derived TRX has been linked to increased virulence whilst microrganism-derived TRX is associated to evasion from the host's immune system. Fungal infections are often present in individuals which are not immune competents such as HIV^+ patients, pregnants and the elderly. In some cases, fungal infections may become systemic, and thus cause sepsis, often leading to multiple organ failure and occasionally death. Here, we investigated the effects of human recombinant TRX on *Candida albicans* virulence *in vitro.* **Methods**: The effects of human TRX (1.0-4.0 µg/ml) were investigated on *Candida albicans* growth, cell viability, metabolism capacity and biofilm formation ability. For this, *Candida albicans* ($1x10^3$ - $5x10^3$ CFU/ml) was cultured in RPMI (pH=7.0) in the presence and absence of human TRX, at 37ºC for 24h. Vehicle-treated *C. albicans* were used as controls. To determine growth, the absorbance was read at 620 nm and taken as growth rate. Cell viability and metabolic capacity, was evaluated by PrestoBlue reagent (Life Technologies) and the results were calculated according to the manufacturer´s instructions. Biofilm formation was quantified by allowing *C. albicans* to grow overnight, at 37ºC in RPMI. Following 24h, the wells were washed 3x, crystal violet was then added to each well and the plate was incubated for 5 min. Methanol was added and incubated for 10 min and the absorbance was read at 620nm. **Results**: TRX decreased *C. albicans* growth when incubated at the concentrations of 1.0 and 2.0 µg/ml (~22%; $p<0.05$). *C. albicans* viability was markedly decreased by 21% ($p<0.05$) when incubated with human TRX (1.0 µg/ml). PrestoBlue metabolism was inhibited by 19% when C. albicans was incubated with human TRX (2.0 µg/ml; $p<0.05$). No significant effects were observed for any of the evaluated concentrations when human TRX was incubated with *C. albicans.* Conclusions: Human TRX presented with inhibitory effects on *C. albicans* growth, viability and metabolism, which may reflect on a reduced virulence of this microorganism. However, more experiments are necessary to further understand the impact these effects may have *in vivo* with host´s immune system fighting *C.albicans* infection. **Financial Support**: This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão (FAPEMA). S.J.F.M. is an MSc student receiving a grant from FAPEMA. B.L.R.S. is an undergraduate student receiving a grant from CAPES.

04.036 Emerging treatment for Psoriasis: **Role for hydrogen sulphide donor, GYY4137.** Rodrigues L[1], Schmidt TP[1], Cerqueira ARA[1], Florenzano J[1], Santos KT[1], Teixeira SA[1], Wood ME[2], Whiteman M[2], Muscará MN[1], Costa SKP[1] [1]ICB-USP – Farmacologia, [2]University of Exeter-St. Luke's

Introduction: Itch is a sensory modality that similar to pain acts as a protective mechanism, and is commonly associated with immune-mediated skin diseases (e.g. psoriasis), where its therapeutic control is poor. In the past few years, H_2S emerged as a novel and important biological mediator associated with various inflammatory conditions. Recently, Alshorafa et al. (Tohoku J Exp Med. 2012; 228(4): 325) demonstrated that psoriatic patients exhibited lower serum levels of H_2S compared to healthy individuals. In addition, our previous work shows that the slow and fast-releasing H_2S donors exerted a protective effect in distinct acute sensory modalities in mice, such as itch and pain (Rodrigues et al. Nitric Oxide. 2013; 31 Suppl 2: 54). **Aims**: We aimed to investigate the efficacy of a daily systemic treatment with the slow releasing-H_2S donor GYY4137 in the severity of psoriasis-like skin index (PASI) and spontaneous itching behavior in a psoriasis-like skin model induced by imiquimod (IMQ), a Toll 7 receptor agonist. **Methods**: Experiments were carried out in male mice (BALB/c; 25-30g) under approved animal license (CEAA/USP; n. 100, book 3, page 09). Mice were anaesthetized with isoflurane, the dorsal skin was shaved and the psoriasis-like disease was induced by daily application of IMQ (5%; 65 mg/animal; Germed Pharmaceuticals, Brazil; n=5) throughout 5 days in the dorsal skin and ear surfaces. Control-treated mice received the equivalent amount of vaseline (65 mg/animal). The severity of PASI was scored daily over six days. Erythema and scaling appearance were scored from 0 to 4, and ear thickness assessed via digital caliper. Spontaneous itching was recorded (Sony Handycam DCR-PJ6, Japan; 30 min) on days 1, 4 and 6. At day 6, animals were euthanized and biological samples collected. Control and psoriatic-like mice were daily treated, via i.p., with GYY4137 (25, 50 or 100 mg/kg) or its vehicle. Stats were performed via ANOVA plus Dunnett's test. **Results**: Imiquimod induced signs and symptoms that closely resembles human plaque type psoriasis, such as erythema/scaling (score: 4/4 vs. 0/0), epidermal thickening, (0.34 vs. 0.21 mm), spontaneous itching (92 vs. 2 scratching bouts) and increased cell influx in peripheral blood (5.4 vs. 3 x 10^6 cells/ml) and spleen (15 vs. 5 x 10^7 cells/ml), but unaffected the levels of antioxidant enzymes. Systemic treatment with GYY4137 significantly inhibited IMQ-induced erythema (score 2.75), scaling (score 3), epidermal thickening (0.32 mm), itching (22 scratching bouts), leukocyte counts in peripheral blood (3.5 x 10^6/ml) and spleen (9.5 x 10^7/ml) and increased antioxidant activities of glutathione peroxidase, reductase and S-transferase. **Conclusions**: We provide the first evidence that systemic administration of GYY4137 greatly reduced signs and symptoms of a psoriasis-like skin model, and this seems to be correlated with the increased skin antioxidant activity. Therefore, exogenous supplementation with H_2S might be of interest to treat psoriasis. **Acknowledgments**: CNPq, CAPES and FAPESP for financial support, and IM Gouvea for technical assistance.

04.037 *In vitro* **LPS-induced zymosan phagocytosis and inflammatory activity of murine peritoneal macrophages are mediated by protease-activated receptor (PAR)2**. Barra A, Siqueira MVA, Matos NA, Freitas KM, Lopes MTP, Klein A ICB-UFMG – Farmacologia

Introduction: PAR2 is a G protein-coupled receptor activated by mast cell tryptase, trypsin, chymase and others through their proteolytic cleavage at a specific site on their N-terminal amino acid sequence, is expressed on the surface of leukocytes and has been implicated in hallmarks of inflammation, however their role in macrophage-mediated phagocytosis and macrophage activity are still unclear. **Aim**: Investigate the role of PAR2 on *in vitro* phagocytosis of zymosan and on inflammatory mediators production in the LPS-stimulated peritoneal macrophages. **Methods**: Macrophages obtained from thioglycolate-injected C57Bl/6 mice were preincubated with LPS (10µg/ml) 30 min prior to PAR2 agonist SLIGRL-NH2 (SLI, 5 or 30µM). The incubation occured in the presence or not with their antagonist ENMD-1068 (ENMD, 30µM), followed by incubation with zymosan (Zy, 10 µg/ml, 1 h). The Phagocytosis index (PI) was defined as the ratio between the total number of phagocytosed Zy particles by percentage of phagocytic cells. The nitric oxide level (NO) was measured by Griess method, reactive oxygen species (ROS) was detected by fluorimetry, and IL-10 and TNF-α; levels determined by ELISA. Statistical analysis were performed using one-way ANOVA followed by Tukey post-test. Experimental procedures were approved by the local animal ethics committee (certificate number 374/2014). **Results**: SLI incubation increases PI (DMEN 4,8 ± 0,3; 5µM 6,5 ± 0,3; 30µM 8,2* ± 1,3; *p<0.01 compared to LPS+Zy), reduces Nitrite production (µM) at 48 h of the culture (Zy 0,7 ± 0,3; LPS + Zy 5,9 ± 0,5; SLI 5µM, 3,2* ± 0,3; SLI 30µM, 3,3* ± 0,6; *p<0.05 compared to LPS + Zy) and these effects on the PI are antagonized by PAR2 ENMD antagonist (DMEN, 2,70 ± 0,167; SLI 30 µM, 6,75 ± 0,15; SLI + ENMD 0,1µM, 3,75* ± 0,25; SLI + ENMD 0,5µM, 2,60* ± 0,2; SLI + ENMD 1µM, 2,62*± 0,53; *p<0.05 compared to SLI 30 µM). ROS production has not been affected by SLI incubation 24 h (DMEM 100 % ± 9,2%; LPS + Zy 82,0% ± 10,3%; SLI 5µM 102,6 % ± 5,7%; SLI 15µM 94,8% ± 8,8%; SLI 30µM 100,6 % ± 26,0%) or 48h after (DMEM 100 % ± 10,3%; LPS + Zy 113,6% ± 9,2%; SLI 5µM 127,2 % ± 10,0 %; SLI 15µM 119,7% ± 10,3%; SLI 30µM 127,7 % ± 9,2%). SLI increases IL-10 production (pg/mL), peaking after 24h of cell culture (DMEM 130,5 ± 11,3; LPS + Zy 692,1 ± 22,6; SLI 30µM 1009,0 ± 58,8*; P<0,01 compared to LPS + Zy), and impaired LPS-induced TNF-α releasing 6 or 24 h after (6h: DMEM, 17,02 ± 2,87; LPS + Zy 569,4 ± 35,5; SLI 30 µM, 492,7 ± 43,7; 24h: DMEM 87,1 ± 8,7; LPS + Zy 393,7 ± 79,5; SLI 30µM 384,0 ± 50,7). **Discussion**: In vitro PAR2 activation was able to increasing phagocytosis and the anti-inflammatory activity of IL-10 released by peritoneal macrophages, in addition, was able to reduce the releasing of the proinflammatory cytokine TNF-α; as well as NO production. Taken together our results suggest a role for PAR2 on the modulating of two important mechanisms of macrophage repertory. **Conclusion**: Our data demonstrate a role for PAR2 on the macrophage activation, suggesting this receptor as a potential target to the treatment of chronic inflammatory diseases. **Financial support**: CNPq, Fapemig.

04.038 Friedelin and Friedelin complexed in cyclodextrin reduces airway allergic inflammation in a murine model of asthma. Ferro JNS[1], Serra MF[2], Santos SL[1], Cotias AC[2], Lima FF[2], Aquino FLT[1], Silva JPN[1], Alves PR[1], Broetto L[1], Ferreira FR[3], Abreu FC[3], Conserva LM[3], Martins MA[2], Barreto E[1] [1]ICBS-UFAL, [2]Fiocruz, [3]UFAL – Química e Biotecnologia

Introduction: Previous investigations revealed that the natural triterpene friedelin has anti-inflammatory and antioxidant properties, but poor water solubility limits its putative clinical application. **Aim**: The aim of this study was to assess the anti-asthma effect of the inclusion complex of friedelin and hydroxypropyl-β-cyclodextrin (HP-β-CD), using a murine model of allergen-induced lung inflammation. **Methods**: The complex was developed via the co-evaporation method mixing friedelin with HP-β-CD in a 1: 2 molar ratio. The formation constant of friedelin-HP-β-CD complex was estimated by the Benesi–Hildebrand method, based on the spectrophotometric quantification of free HP-β-CD at 228 nm and 278 nm. Mice of strain A/J and Swiss (n=6/group) were sensitized with ovalbumin (OVA) and then treated with intranasal instillation of friedelin (24.3 µmol/Kg) or friedelin: HP-β-CD (24.3 µmol/kg), 1 h before OVA challenge. Analyses were carried out at 24 h post-OVA provocation. Leukocytes recovered from the bronchoalveolar lavage (BAL) effluent were stained and enumerated via cytospin preparations. Lung fragments were collected for histopathology by H&E and Gomori staining, quantification of cytokines levels by ELISA and mRNA expression of IL-4 by RT-PCR. **Results**: The increased levels of total leukocytes in BAL fluid reduced from $63x10^4$ cells to $14x10^4$ cells and $9x10^4$ cells, following friedelin and friedelin complex, respectively. In case of eosinophil counts, values reduced from $38x10^4$ cells to $5x10^4$ cells and $3x10^4$ cells, respectively. In lung from OVA-challenged mice the treatment with friedelin and friedelin-HP-β-CD inhibited the levels of IL-4 in 20% and 41%, respectively, while the levels of eotaxin-1 were reduced in 41% and 49%, respectively. Friedelin and friedelin complex significantly inhibited the levels of IL-17 (45% and 65%), KC (50% to 68%), MIP-1α (3% to 62%), TARK (67% to 78%), and IL-13 (51% to 70%) in the lung tissue. Furthermore, friedelin and friedelin-HP-β-CD reduced in 16% and 62% the expression of IL-4 mRNA in lung from asthmatic mice, respectively. In addition, friedelin and friedelin complex inhibited OVA-induced accumulation of inflammatory cells and extracellular matrix in peribronchiolar area. Remarkably, friedelin and friedelin-HP-β-CD increased in 25% and 43% the levels of IL-10 in the lung tissue after OVA challenge, respectively. Treatment with HP-β-CD alone did not show any anti-inflammatory effect. **Conclusions**: These results suggest that both friedelin and friedelin-HP-β-CD, administered intranasal, can prevent pivotal features of lung allergic response, but the latter seems to present a better efficacy, probably due to a superior bioavailability. The protective effect of friedelin on asthma inflammatory changes appears to be mediated by IL-10 production. **Financial support**: CNPq, CAPES. UE FP7- 2007-2013 - grant agreement HEALTH-F4-2011-281608. CEUA/UFAL (License 044/2013) and CEUA/Fiocruz (License no LW-23/10).

04.039 Anti-inflammatory effect of low-level laser therapy and the role of nitric oxide in carragenan induced edema. Cruz JSJ, Mazulo JCRN, Sousa NA, Queiroz FFSN, Brito TV, Barbosa ALR, Filgueiras MC UFPI – Acadêmico

Introduction: Low-Level Laser Therapy (LLLT) promotes photobiomodulation inflicting on inflammatory process, tissue repair, chronic pain and infections. Nitric Oxide (NO) is a gas that induces vasodilatation and play as a messenger between and intra cellular, also mediating an analgesic effect caused by LLLT. However, there are no studies showing its anti-inflammatory action in acute paw edema (Karu *et al*., 2005; Moriyama *et al*., 2009). **Objective**: To investigate the role of NO over the effects of the LLLT in acute paw edema and myeloperoxidase. **Methods**: In the assays involving carrageenan (Cg; 500µg/paw) induced paw edema, mice (groups n = 6): group1, Cg; group2, Cg + aminoguanidine + L1; group3, Cg + aminoguanidine + L-arginine + L1; group4, salina into the paw, were treated 30 min before each mensuration with low-level laser (L1 J/cm²) or not treated (control group). The study was approved by Ethics Committee of the Faculdade Integral Diferencial—FACID, number protocol: 002/13. Paw volume measured in a plethysmometer immediately before injections and then 1, 2, 4 and 6 h later. The animals sacrifice in the sixth hour and paw tissue of the right were remove for measurement of MPO. The results were report as the MPO units/mg tissue. **Results**: Administration of carrageenan (500µg/paw) in paw, promoted the formation of edema 1 hour after injection and was gradually heightened, with a peaking at 4^{th}h (0,091 ± 0,003 ml). Laser in dosimetry of 1J/cm2 applied to the plantar region during 12s always 30 minutes before measuring the paw volume, decreased edema in all time points measured, including the 4^{th}h (0,060 ± 0,005ml). In the test to verify participation of NO in the LLLT, carrageenan increased the paw volume peaking in 4^{th}h (0.067 ± 0.007 ml). Laser decreased edema significantly (4^{th}h: 0.028 ± 0.006 ml). The effect was lost with aminoguanidine, a nonspecific blocker of NOS (4^{th}h: 0.076±0.008 ml). L-arginine (a donator of NO) diminishes the edematogenic condition (4^{th}h: 0.050 ± 0.008 ml). Myeloperoxidase assay in positive group (387.1 ± 44.46 UMPO/mg) and the group treated with laser (1 J/cm^2) reduced MPO activity (116.1 ± 13.34 UMPO/mg). Aminoguanidine intensified MPO activity (283.3 ± 30.95 UMPO/mg). L-Arginine reduced MPO activity (99.17 ± 27.17 UMPO/mg). **Conclusion**: This study we can infer that Low-Level Laser Therapy (LLLT) seems to have a relation with synthesis and release of NO in the reduction process of neutrophil migration and consequently decreases the edematogenic condition during carrageenan-induced inflammation. **Financial support**: CNPq and FAPEPI Karu *et al*. Cellular Effects of Low Power Laser Therapy Can be Mediated by Nitric Oxide. Lasers in Surgery and Medicine. n. 36 p. 307–314, 2005. Moriyama *et al*. In Vivo Effects of Low Level Laser Therapy on Inducible. Nitric Oxide Synthase. Lasers in Surgery and Medicine. n. 41, p. 227–231, 2009.

04.040 Antinociceptive, antiedematogenic and anti-inflammatory effects of *Borreria verticillata* and its compounds. Teixeira FM[1], Ferreira RT[1], Guimarães LD[2], Silva RF[1], Malvar DC[1], Chaves DAS[2], Vanderlinde FA[1] [1]UFRRJ – Ciências Fisiológicas, [2]UFRRJ – Química

Introduction: *Borreria verticillata* (BV), or "vassourinha de botão", belongs to the family Rubiaceae, is a folk medicinal plant used as antipyretic, analgesic and anti-inflammatory. **Aim**: This study evaluated the antinociceptive, antiedematogenic and anti-inflammatory activities (*p.o.*) of leave extract from BV (LEBV) and its flavonoidic (FFBV), butanolic (BuFBV) and acetate (AcFBV) fractions. **Methods**: Groups (n=8) of male Swiss mice (25-35g) were pretreated by oral route and the results were expressed as mean±S.E.M. Writhing test: after 30 minutes of treatments with LEBV (100, 300 or 1000mg/kg) or indomethacin (10mg/kg), the number of writhes induced by acetic acid injection (1,2%, *i.p.*) was counted (30min). Ear edema: 30 min post treatment with LEBV (100, 300 and 1000mg/kg) or Dexamethasone (Dexa) (2mg/kg), edema was induced (croton oil, 2.5%, right ear surface). After 4 hours, the weight difference (Δ) between ears was measured. Pleurisy test: after 1 hour of treatment with LEBV, FFBV, BuFBV, AcFBV or Dexa (2mg/kg), the pleurisy was induced (carrageenan, 1%, intrapleural). After 4 hours, the number of leukocytes (LK) was counted. Arthritis: 1 hour after treatment with LEBV (1g/kg) or dexa (2mg/kg), arthritis was induced by zymosan (15µg/µL; intraarticular). After 4 hours the edema and LK migration were evaluated. **Results**: LEBV (100-1000mg/kg), produced a dose related antinociceptive effect in writhings (w) by 23.7% (30±2.9w), 30.2% (27.4±2.5w) and 47.3% (20.7±4.1w) respectively, while indomethacin reduced writhings by 54.7% (17.8±1.8w) comparing with vehicle (39.3±2.9w). Only the higher dose of LEBV reduced ear edema by 47.8% (Δ=1.78±0.3mg), while dexa reduced by 66.9% (∆=1.1±0.3mg), comparing with vehicle (Δ=3.4±0.2mg). In pleurisy, LEBV (1g/kg), FFBV (20mg/kg), BuFBV (200mg/kg), AcFBV (25mg/kg) or dexa, reduced LK migration by 33.7% (4.5±0.5 x 10^6LK/mL), 74.8% (2.5±0.8 x 10^6LK/mL), 71% (3.4±0.4 x10^6 LK/mL), 79.1% (2.5±0.3 x10^6 LK/mL) and 85.5% (1.7±0.2 x 10^6LK/mL) comparing with its respective vehicles. LEBV and dexa reduced LK migration to knee articulation by 65% (1.4±0.4 x 10^6LK/mL) and 90% (0.4±0.1 x 10^6LK/mL) comparing with vehicle (4.0±0.6 x 10^6LK/mL). Simultaneously, the articular edema was reduced by 58.8% (0.7±0.1mm) and 82.3% (0.3±0.2mm), respectively, comparing with vehicle (1.7±0.2mm). **Conclusion**: These results may explain some popular antinociceptive and anti-inflammatory indications of this species. **Financial support**: CNPq. Methodology approved by COMEP-UFRRJ (nº2427).

04.041 Nanocapsules increase alpha-bisabolol bioavailability in lung tissue and reduce acute pulmonary inflammation induced by LPS in mice. D'Almeida APL, Ciambarella BT, Souza ET, Terroso T, Coutinho DS, Gomes CR, Oliveira NS, Pohlmann AR, Guterres SS, Silva PMR, Martins MA, Bernardi A Fiocruz – Inflamação

Introduction: Alpha-bisabolol is a sesquiterpene alcohol obtained of essential oil from plants with an anti-inflammatory activity. Nanotechnology enables drug vectorization that leads to higher bioavailability in the target tissue. This work aims to evaluate the anti-inflammatory effect of alpha-bisabolol-loaded nanocapsules (α-bis NC) on pulmonary inflammatory model LPS-induced in mice. **Methods**: Mice were treated with free alpha-bisabolol (α-bis) or α-bis NC (30, 50, 100 mg/Kg, p.o.) or drug-unloaded nanocapsules (NC) (100 mg/Kg p.o) 4 h before saline (25 µl/animal, i.n.) or LPS provocation (25 µg/25 µl, i.n.). After 18 h parameters of acute lung injury were monitored. Airway hyper-reactivity (AHR) and pulmonary elastance were assessed by invasive whole-body plethysmography using increasing concentrations of aerosolized methacholine. Total and differential leukocytes were quantified in bronchoalveolar lavage fluid (BALF), with the Neübauer chamber and cytocentrifuged slides stained with May-Grünwald-Giemsa, respectively. Pro-inflammatory chemokines were quantified in lung tissue samples using ELISA. The indirect amount of infiltrating neutrophils was estimated by myeloperoxidase (MPO) activity assay. Quantitative analyses by HPLC were performed to determine the pulmonary bioavailability of α-bis carried by polymeric nanocapsules, compared to free α-bis. **Results**: We found that α-bis NC (30, 50 and 100 mg/Kg) significantly reduced LPS-induced AHR concerning to lung elastance (54%, 61% and 50%, respectively) when compared with NC treated mice. In the BALF the total number of leukocytes was increased in NC treated and LPS-induced group when compared to saline group. Again, α-bis NC (30, 50 and 100 mg/Kg) significantly reduced LPS-induced elevation in total leukocyte (756.4, 551.2 and 549.8, respectively; $p<0.05$; mean $x10^3$) and neutrophil (109, 37.4 and 26.7, respectively; $p<0.0001$; $x10^3$) numbers, when compared with the NC treated group (892.8 and 805). Remarkably, α-bis NC (30, 50 and 100 mg/Kg) presented a blockade of neutrophil recruitment, which was significantly higher compared with that noted following the respective free-treated doses (781.3 to 109, 807.3 to 37.4 and 675 to 26.7, respectively; $p<0.001$). The MPO activity was reduced in animals treated with α-bis (100 mg/kg) (0.40; U/mg protein) and α-bis NC (30, 50 and 100 mg/kg) (0.04, 0.05 and 0.04, respectively; p<0,0001; mean). Moreover, α-bis NC (30, 50 and 100 mg/Kg) reduced the levels of KC (36.77, 16.44 and 21.59, respectively; pg/mg protein; $p<0.05$), MIP-1 (10.62, 7.48 and 9.96) and MIP-2 (695.2, 1129 and 794.5). HPLC quantification showed that animals treated with α-bis NC presented 6- (30 mg/Kg), 20- (50 mg/kg), and 16- (100 mg/kg) fold higher α-bis concentrations in the lung, than those treated with respective doses of free α-bis. **Conclusion**: In summary, the data reported here demonstrated that polymeric nanocapsules are able to successfully carry α-bis into the lung tissue and local delivery of α-bis reduced LPS-induced pulmonary inflammation. The elucidation of mechanism of action of α-bis on LPS-induced pulmonary inflammation is in progress. **Financial support**: CNPq, FAPERJ. **Ethics Committee of Animal Use**: CEUA - LW23/10.

04.042 Reduced lung inflammation in intrauterine undernourished rats is not related to high circulating levels of corticosterone. Gil NL[1,2], Azevedo G[2], Silva MM[2], Fernandes L[2], Landgraf MA[3,2], Landgraf RG[2] – [1]ICB-USP – Imunologia, [2]Unifesp-Diadema – Inflamação e Farmacologia Vascular, [3]ICB-USP – Farmacologia

Introduction: We observed that intrauterine undernourished rats (UR) presented a decrease in inflammatory response that could be related to the increased levels of glucocorticoids. In the present study we evaluated if decreased inflammatory response observed in these rats could be reversed by adrenalectomy and replacement physiological levels of corticosterone. **Materials and Methods**: Intrauterine nourished and undernourished (50% food restriction) male rats, adrenalectomized (ADX), were replaced with corticosterone (i.p., 3mg/kg/day) or saline for seven consecutive days. Then, the acute lung injury induced by LPS intratracheal instillation *(750□g/200□L)* was evaluated: the bronchoalveolar lavage fluid was collected and cellular infiltration into lung tissue was analyzed. Lungs were harvested for measurement of cytokine release (multiplex) and analysis of glucocorticoid receptor expression (western blot). **Results**: We did not observe difference in cell infiltration and cytokine levels in ADX group. Undernourished rats stimulated by LPS presented significantly reduced in total cell (42.8%) and neutrophils (63%) in bronchoalveolar lavage and lung tissue. Increased TNF-α(69.8%) and IFN-□(71.8%) expression when compared to nourished group but no difference was observed in IL-1□ and IL-6 levels. IL-2 levels were increased in ADX+LPS groups but did not differ between nourished and undernourished. Western blot assay showed that expression of glucocorticoid receptor is reduced in undernourished, but adrenalectomy, increased glucocorticoid receptor expression only in undernourished offspring. **Conclusion**: Our preliminary results indicate that high circulating levels of corticosterone is not related to reduced inflammatory lung response in intrauterine undernourished rats. **Supported by** FAPESP-2012/51104-8, 2010/01404-0, CNPq and CAPES. **Animal Research Ethical Committee**: (CEUA 76/2014)

04.043 Anti-inflammatory and antinociceptive activity evaluation of oleoresin of *Copaifera reticulata* in animal model. Almeida Jr J, Silva EBS, Moraes TMP, Oliveira ECP, Moraes WP ISCO-UFOPA

Introduction: The *Copaifera reticulata*, popularly known as "capaibeira" buds out oleoresin from its stem when there is drilling. The exudate is often used in folk medicine, especially based on its anti-inflammatory and antinociceptive properties. OBJECTIVE: To evaluate the antinociceptive and anti-inflammatory activity of oleoresin, obtained from *Copaifera reticulata* originated from Tapajos National Forest. The acute toxicity was also analyzed. **Methods**: The oleoresin of *Copaifera reticulata* (OCR) was collected in the Tapajós National Forest during the dry season and characterized by the Biotechnology laboratory of Plant at UFOPA. Wistar rats (150 - 200 g) and Swiss mice (25 - 35g) were used. All the experimental procedures were approved by the Animal Ethics Committee in UFOPA under the number 7004/2013. Acute toxicity was held to establish the doses and OCR security level tested according to the protocol 423 of the Organization for Economic Cooperation and Development in 2001 (OECD). For the antinociceptive activity the hot board method was used, which evaluates the central analgesia through the determination of latency time for the animals to respond to the heat stimulus. For the antinociceptive activity, the abdominal writhing test was used, which measures the number of stereotypical reactions of contractions of the abdominal walls triggered by 0.6% acetic acid intraperitoneal injection. For the anti-inflammatory and antiedamatogenic activity, paw edema and air pouch systems were used, under condition of intraplantar or subcutaneous injection of 1% carrageenan stimulation. **Results**: In the acute toxicity analysis, it was determined that the toxic dose of OCR is above 2000 mg/kg. OCR did not alter the latency time when in the hot board. However, OCR inhibited the number of of writhings, at all three doses - 10, 100 and 400 mg/kg. The evaluation of the antiedematogenic activity showed that OCR, at a dose of 100 mg/kg, reduced paw edema at all times. The OCR was also able to reduce the number of infiltrated leukocytes and the volume of exudate of the air pouch system. in all three doses tested when evaluated by air bag method. **Conclusions**: Treatment with OCR had no antinociceptive central effect, but had an effect on the periphery. In line with these results, we showed that OCR had anti-inflammatory and antiedematogenic activity, as attested by reduction of paw edema and leukocyte recruitment to the inflamed site.

04.044 Heparan sulfate (HS) inhibits the synthesis of melatonin in rat pineal glands via toll-like 4 receptors (TLR4) activation. Acco M[1], Cecon E[2,1], Nader HB[3], Markus RP[1] [1]USP – Fisiologia, [2]Institut Cochin, [3]Unifesp – Bioquímica

Introduction: The nocturnal synthesis of melatonin by the pineal gland is blocked by the interaction of pathogen-associated molecular patterns with TLR4 (Markus et al, Int. J. Mol. Sci.14, 10979, 2013). Nuclear translocation of NF-kB dimers blocks noradrenaline (NAd)-induced transcription of the gene that codes the enzyme that converts N-acetylserotonin to melatonin. The reduction in nocturnal plasma level of melatonin favors migration of leukocytes to injured tissues. In addition, when pineal gland activity declines, spurious cell migration favors a low-grade inflammatory state. Heparan sulfate (HS) disaccharides released from proteoglycans by the disruption of the extracellular matrix are endogenous ligands for TLR4 (Brunn et al. FASEB J. 19, 872, 2005). Here we evaluated whether HS blocks the synthesis of melatonin in cultured pineal glands by activating TLR4. **Methods**: Pineal gland of female Wistar rats (45 days) kept in light / dark cycle 12/12h, were removed and maintained in culture for 48 hours. After denervation, the synthesis of melatonin was induced by noradrenaline (10nM, 5h). HS (0, 0.3, 1, 3, 10 or 30 µg/mL) was pre-incubated for 30 min, and maintained till the end of noradrenaline incubation. TLR4 receptors were blocked with TAK-242 (1nM, 30 min) previously to HS and noradrenaline incubation. The content of melatonin was determined by high-performance liquid chromatography. **Results**: HS decreased melatonin content in the medium in a dose-dependent manner (noradrenaline-induced content = 799.5±67 ng/mL (n=7); maximal HS reduction = 50%). TLR4 competitive antagonist reversed the HS inhibition of noradrenaline-induced melatonin synthesis. The level of melatonin precursors was not modified by HS. **Discussion**: HS via TLR4 modulates the synthesis of melatonin in rat pineal glands. As observed for other TLR4 agonists (lipopolysaccharide, zymosan and □-amyloid peptide) HS is inhibiting the conversion of N-acetylserotonin to melatonin. These data suggest that the increase in free HS in pineal gland matrix is translated to the whole organism by a reduction in the nocturnal melatonin peak. **Financial Support**: FAPESP (2013/15037-7). CEUA IB-USP License 048/2007, 198/2014 and ICB-USP number 105/2013.

04.045 Effects of augmented O-Glcnacylation on activation and differentiation of macrophages. Zanotto CZ, Olivon VC, Mestriner FLAC, Alves-Filho JC, Carneiro FS, Tostes RC FMRP-USP – Farmacologia

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

04.046 Investigation of a nanodispersion system and its impact on skin delivery of the hydrogen sulfide donor (GYY4137) in an experimental model of psoriasis.

Schmidt TP[1], Rodrigues L[1], Cerqueira ARA[1], Carvalho VFM[1], Teixeira SA[1], Wood M[2], Whiteman M[2], Muscará MN[1], Lopes LB[1], Costa SKP[1] [1]ICB-USP – Farmacologia, [2]University of Exeter-St. Luke's

Introduction: Pruritus is an important symptom in psoriasis, an immune-mediated skin disease, with no effective topical treatment. Recently, we showed that systemic administration of GYY4137, a slow-releasing hydrogen sulfide (H_2S) donor is an effective treatment for experimental imiquimod (IMQ)-induced psoriasis and associated pruritus in mice (Rodrigues et al., Nitric Oxide, 47: S35, 2015). However, the inconvenience of the systemic administration is a challenge to establish a safe and potential use in psoriasis. In order to improve the use, safety and efficacy of GYY4137 in psoriasis and related pruritus, we questioned how to develop a GYY4137 nanodispersion system for topical treatment of psoriasis, with improvement in cutaneous absorption and local effect. **Aim**: We evaluated a dose-response daily regimen of GYY4137 nanodispersion in the treatment of experimental IMQ-induced psoriasis. **Methods**: Under isoflurane anaesthesia, the dorsal skin of BALB/c mice was shaved (6 cm^2) and psoriasis was induced by the topical application of the TLR-7 receptor agonist IMQ (5% cream; 65 mg/animal; Germed, Brazil), on the dorsal skin and ear surface throughout 5 consecutive days. Control group received an equivalent amount of vaseline. Nanodispersion was prepared by mixing Poloxamer 1% with GYY4137 incorporated, oleic acid and monolein. Mice with psoriasis were treated with GYY4137 nanodispersion at 1, 2 or 4%, or its vehicle (poloxamer + oleic acid and monolein 9: 1 w/w; 65 mg/day). The severity of psoriasis was scored daily throughout 6 days. The spontaneous itching behaviour was recorded for 30 min at days 1, 4 and 6, when myeloperoxidase (MPO) activity was also evaluated. At day 6, the mice were euthanized and skin collected to proceed with biochemical analysis. **Results**: The single daily topical administration of the GYY4137 nanodispersion greatly reduced, in a non-dependent concentration fashion, the increased MPO (7.9 ± 2.3 U/mg for psoriasis and 1.2 ± 0.4, 1.9 ± 1.1 and 1.6 ± 0.9 U/mg for psoriasis plus GYY4137 at 1, 2 and 4% concentrations respectively; n=7-9), the increased IL-6 on skin (20.45 $\pm$ 6.60 pg/mg for psoriasis and 3.0 $\pm$ 2.31 and 4.54 $\pm$ 3.19 pg/mg for psoriasis plus GYY4137 at 2 and 4% concentrations respectively; n=5-8) and partially inhibited erythema score (7.2 ± 0.7 for psoriasis and 4.5 ± 0.3 AUC for psoriasis plus GYY4137 at 4% concentration; n=7-9), but only slightly reduced scaly skin, ear thickness, IL-1β levels on skin and spontaneous itching, compared to untreated mice with psoriasis. **Conclusion**: Although daily treatment with the nanodispersion containing GYY4137 for 5 days is partially effective for most signs and symptoms of IMQ-induced psoriasis in mice, intensification of dosing to twice daily with this formulation (65 mg) might be necessary to elicit a full effective response, including pruritus. **Financial support**: FAPESP (grant #2014/15576-8), CAPES and CNPQ. ETHICAL ASPECTS: Experiments were carried out under approved animal protocol use (CEAA/USP; n100, b3, p9). ACKNOWLEDGEMENTS: IM Gouvea and AG Soares for technical assistance.

04.047 Proteolytic fraction from *Vasconcellea cundinamarcensis* **latex stimulates macrophage activity against inflammatory breast cancer cells**. Braga AD[1], Freitas KM[1], Teixeira LCR[1], Salas CE[2], Lopes MTP[1] [1]ICB-UFMG – Farmacologia, [2]ICB-UFMG – Biochemistry and Immunology

Introduction: Previous studies demonstrated that a proteolytic fraction (P1G10) from *V. cundinamarcensis* latex has antitumor/antimetastatic activity upon 4T1 breast carcinoma model by reducing inflammation, angiogenesis and increasing tumor associated-macrophages (TAMs) activity. TAM's M2 phenotype can promote angiogenesis, remodeling of extracellular matrix and invasion of tumor cells. In contrast, M1 phenotype are capable to kill tumor cells and promote immune antitumor response[(1).] Our data shown that macrophages exposed to P1G10 are capable to reduce 4T1 cells number in co-culture model. **Aim**: Investigate the ability of P1G10 proteolytic sub-fractions (CMS1 and CMS2) to promote macrophage cytotoxic phenotype and its activation mechanisms. **Methods and Results**: Balb/c female mice received thioglycollate (3%, *i.p.,* 2 mL). In 3^{thr} day, macrophages were harvested by peritoneal laved, seeded in 24 wells plates (3x10^5 cells/well) and exposed to CMS1 or CMS2 (10, 20 or 40 µg/mL) for 24 hs. Then treatments were removed and co-cultures made by plating 4T1 tumor cells (1x10^5 cells/well). After 40hs, MTT test was realized and 4T1 cells number expressed as Δ D.O. at 570nm (D.O. co-culture – D.O. macrophage culture). Macrophages pre-exposed to CMS1 were not able to promote tumor cells death (0.324 ± 0.037 – 0.394 ± 0.021 *vs* 0.362 ± 0.015 ΔD.O. –control). By the other hand, macrophages pre-exposed to CMS2 (20 and 40 µg/mL) reduced 4T1 cell number in 40 and 57%, respectively (0.217 ± 0.022 and 0.155 ± 0.014 *vs* 0.362 ± 0.015 ΔD.O. – control, p<0.0001). To investigate with reactive oxygen or nitrogen species (ROS or RNS) are involved in this activation effect, macrophages were seeded in 96 well plates(1x10^5 cells/well) and exposed to CMS1 or CMS2 (5-40 µg/mL) for 24hs, when were labeled with DCFH-DA (15µM) for 30 min. The release of fluorescence at 480_{Ex}–530_{Em} nm was measured as ROS production. CMS1 (30 and 40 µg/mL) increased ROS production until 104 % (10,339 ± 166 and 17,596 ± 654 *vs* 8,613 ± 103 ΔFU/ cell number – control p< 0.0001). Although, CMS2 (10- 40 µg/mL) increased until 171% (18,802 ± 611 – 28,904 ± 1,195 *vs* 10,647 ± 331 ΔFU/ cell number – control, p< 0.0001). The production of nitric oxide (NO) was determined indirectly, using Griess method, trough quantification of nitrite in macrophage culture supernatant (540 nm). CMS1 (20-40 µg/mL) increased (131- 969%) NO production (0.167± 0.015 – 0.770 ± 0.039 *vs* 0.072 ± 0.017 µM Nitrite/cell number – control, p< 0.0001). The sub-fraction CMS2 in the same concentrations increased in 150 – 2,249% the nitrite levels (0.238 ± 0.032 – 2.229 ± 0.102 *vs* 0.095 ± 0.017 control – p< 0.0001). Differences between groups were performed using the one-way ANOVA test followed by Student-Newman-Keuls post-test. **Conclusion**: The results suggest that ROS and NO, two kind of molecules that can promote tumor cell death, are involved in macrophage cytotoxic activity induced by P1G10. This effect probable occurs trough proteins presented in CMS2 sub-fraction. **Financial support**: CNPq, Capes, Fapemig. **Ethical committee number**: 219/2012. **References**: (1) SICA et al., 2008. Cancer Letter, 267: 204–215.

04.048 Antimicrobial activity and biochemical and structural analyses of Dermcidin-1L (DCD-1L) and its splice variant (DCD-SV) in biomimetic membranes. Bronze F[1], Riske K[2], Brandão V[2], Belizario J[1] [1]ICB-USP – Farmacologia, [2]Unifesp – Biofísica

Dermcidin (DCD) is produced by epithelial cells of sweat glands of the skin as 110 amino acids precursor protein, which is proteolytically processed to give rise to multiple antimicrobial peptides. The 48-amino acid C-terminal peptide (DCD-1L) has a net negative charge (-2) and DCD-spliced variant (DCD-SV) of 59-amino acids has a neutral charge. We found that DCD-SV peptide possesses higher antimicrobial activity against *E.coli* (Gram negative) and DCD-1L against *S. faecalis (*Gram positive). Next, we evaluated the permeability properties of DCD-1L and DCD-SV synthetic peptides on giant unilamellar vesicles (GUVs) made of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC). DCD-SV peptide promoted rapid increases of permeability of GUVs, in a dose (5-40 □M) and time- (1-5 min) dependent. The structural preferences of peptides were analyzed by circular dichroism (CD) spectroscopy. CD spectra showed that DCD-1L and DCD-SV acquired □-helix secondary structures in the presence of Zn^{2+} (200 □M) and POPC (200 □M). Previous studies have shown that DCD-1L can form ion channels in biomimetic membranes. Our results suggest that DCD-SV peptide has higher propensity to adopt helicoidal structure enabling it to insert into biomimetic membranes and undergo oligomerization. These findings suggest that mechanism of action of DCD-SV is similar of DCD-1L and that both may act synergistically in human sweat to control pathogenic bacteria growth.

04.049 Irinotecan increases regulatory T Cells and Th17 cells in intestinal mucositis. Fernandes C, Wanderley CWS, Muniz HA, Silva CMS, Teixeira MA, Souza NRP, Cândido AGF, Ribeiro RA, Lima-Júnior RCP UFC – Fisiologia e Farmacologia

Introduction: Intestinal Mucositis (IM) is a common side-effect associated with irinotecan (IRI)-based therapy for colorrectal cancer. IM can cause delayed chemotherapy cycles, dose reductions and treatment interruption. The involvement of inflammatory mediators, such as TNF-α, IL1-b, IL-18 and KC, has been demonstrated by our group. However, in the context of IM, the role of adaptive immune system cells (Treg and Th17), whose activation is partially regulated by those cytokines, is yet to be shown. **Aim**: To identify the frequency of Tregs and Th17 cells in IRI-induced Intestinal Mucositis. **Methods**: C57BL/6 mice (20-25g) were injected with saline (n=10) or IRI (75 mg/kg, i.p.), once a day for 4 days. The mice were euthanized at day 1 (D1, n=4), 3 (D3, n=6), 5 (D5, n=4) or 7 (D7, n=4) following the first dose of IRI. Intestinal lamina propria and splenic lymphocytes were harvested by enzymatic and mechanical digestion and were purified by Percoll gradient. The frequency of Tregs and Th17 cells was identified by flow cytometry. In addition, blood leukocyte count ($x10^3$/ml) was obtained and ileum samples were collected for histopathological analysis and myeloperoxidase assay (MPO, neutrophil/mg tissue). ANOVA/Bonferroni's test was used for statistical analysis. *P*<0.05 was accepted. **Results**: IRI-injected mice presented a marked leukopenia [D5: 1.4±0.2; (P<0.01) and D7: 2.0± 0.5; (P<0.05)] and diarrhea in the D7 [2(2-3)] vs Saline group [leukopenia: 5.5±1.2; and diarrhea: 0(0-1)] (P<0.01). Intestinal Tregs frequency ($CD3^+CD4^+CD25^+FOXP3^+$) was also increased on D7 (18.7± 3.1%) when compared with saline (3.6±1.1; P<0.0001), D1 (2.5± 0.6; P<0.001), D3 (5.0± 1.0; P<0.0001) or D5 (8.7±0.9; P<0.01). The same was seen to intestinal Th17 cells (% of $CD3^+CD4^+CCR6^+RORgt^+$ cells) in the D7 (28.2±4.2) versus saline-injected mice (10.9±1.9; P< 0.01). In the spleen, the frequency of Th17 cells (7.3± 1.4) was increased on D7 in comparison to saline (3.8± 0.6; P<0.001), D1 (4.9± 1.0; P< 0.05), D3 (4.2±0.6; P<0.001) or D5 (4.2± 1.0; P<0.01). In addition, neutrophil infiltration (MPO) was increased in the D5 (21171±5779) when compared to saline (3098±1022; P<0.001), but decreased in the D7 (6163±1757; P<0.05 vs D7). Conclusions: These preliminary results suggest that Tregs and Th17 cells might be involved in the pathogenesis of irinotecan-induced IM. Further experiments are needed in order to confirm this hypothesis. Financial Support: CNPq/CAPES/FUNCAP. Animal Research Ethical Committee: 75/2013.

04.050 Down-regulation of single immunoglobulin Interleukin-1R-related molecule (SIGIRR) gene expression during irinotecan-induced intestinal mucositis. Wanderley CWS, Silva CMS, Fernandes C, Muniz HA, Aguiar MG, Lima GS, Wong DVT, Lima-Junior RCP[1], Ribeiro RA[1] [1]UFC – Farmacologia e Fisiologia

Introduction: Severe diarrhea (15-25% of patients) and the associated intestinal mucositis (IM) are common side-effects of colorectal anticancer therapy with Irinotecan (IRI). Currently, there is no effective treatment for IM. However, recent studies suggest the involvement of interleukin-1/Toll-like superfamily of receptors (IL-1R/TLR) in the pathogenesis of IM, which can be triggered by cytokines, such as, IL-1, IL-18, IL-33. The Single Immunoglobulin Interleukin-1R-Related-molecule (SIGIRR) is a new member of the IL-1R/TLR family, which does not elicit a canonical pro-inflammatory signaling cascade. The literature reports that SIGIRR mitigates inflammation triggered by IL-1/TLR receptors through intracellular crosstalk. Furthermore, the anti-inflammatory potential of SIGIRR has been documented in experimental colitis, cancer and rheumatoid arthritis. **Aim**: This study aimed to evaluate the SIGIRR, IL-18, and IL-33 gene expression in early and late phases of irinotecan-induced intestinal mucositis. **Methods**: C57BL/6 mice (n=7) were administered with saline or IRI (75 mg/kg, i.p./4 days). On the 1st, 3rd, 5th, 7th and 14th days after the first dose of IRI, diarrhea scores, body weight, leukocyte count were assessed. In addition, ileum samples were used to measure the myeloperoxidase activity, morphometric analysis, and mRNA expression of SIGIRR, IL-18, and IL-33. Statistical analysis was performed with Kruskal Wallis/Dunn's test or ANOVA/Bonferroni's test as appropriate. $P<0.05$ was accepted. **Results**: IRI increased the gene expression of IL-18 and IL-33, mainly on the 5th and 7th day *vs*. saline group (Day 5: 2.7 ± 0.3 *vs.* 1.0 ± 0.2; Day 7: 6.7 ± 1.8 *vs.* 1.0 ± 0.1, respectively). Gene expression of these cytokines was accompanied by severe diarrhea, loss of body weight, leukopenia, reduction of villus height and increased MPO activity *vs.* saline group (1 [0-3] *vs.* 0 [0-0]; 73 ± 2.6 *vs.* 105 ± 3.3 %; $1.7x10^3$ ± 0,3 *vs.* $7.9x10^3$ ± 1.1; $4.6x10^3$ ± $0.9x10^3$ *vs.* $1.8x10^3$ ± 0.4 $x10^3$, respectively). However, on the late phase (14th day) these parameters returned to basal levels. Interestingly, the expression of SIGIRR was reduced in the acute phase of IM (7th day, IRI: 0.8 ± 0.1 *vs.* saline: 1.1 ± 0.2), but increased in the late phase (14th day, IRI: 1.8 ± 0.2 vs. saline: 1.1 ± 0.2). **Conclusions**: SIGIRR is inversely expressed when compared with IL-18 or IL-33 during early inflammatory and late non-inflammatory phases of intestinal mucositis. The importance of these findings are to be investigated. **Financial support**: CNPq, CAPES and FUNCAP. (CEPA 103/2014).

04.051 Evaluation of *in vivo* **and** *in vitro* **anti-inflammatory activity of** *Rubus imperialis* **extract and the isolated compound Niga-Ichigoside F1.** Tonin TD, Machado ID, Niero R, Petreanu M, Santin JR USP – Análises Clínicas e Toxicológicas

Introduction: *Rubus imperialis* Cham. Schl. (Rosaceae) is frequently used in traditional medicine as hypoglycemic, antinociceptive and antiviral treatment. Previous phytochemical studies carried out with *R. imperialis* have demonstrated the presence of triterpenes (niga-ichigoside F1 (NI-F1) in this species. The literature indicates that triterpenes are closely related to some pharmacological activities, including anti-inflammatory activity. **Aim**: The study was conducted to evaluate the *in vivo* and *in vitro* anti-inflammatory effects of methanolic extract obtained from aerial parts of *R. imperialis* and the compound isolated NI-F1. **Methods**: Male Balb/c mice were orally treated with *R. imperialis* (100 mg/kg) extract or NI-F1 (100 mg/kg) and inflammation was induced one hour later by lipopolysaccharide or carrageenan injection into the subcutaneous tissue (air pouch model). Four hours later, the exudates were collected for leukocyte count and differentiation. Blood samples also were collected to hematological analysis. *In vitro* actions of the extract and NI-F1 were investigated on neutrophils obtained from peritoneal cavity of Balb/c mice (4h after 1% oyster glycogen solution injection; 10 mL), and incubated with vehicle, extract (1, 10 or 100 µg/mL) or NI-F1 (1, 10 or 100 µM) in presence or absence of lipopolysaccharide from Escherichia coli (LPS, 5 µg/ml). In the supernatant was measured the nitric oxide (NO; Griess reaction). In addition, the effect of extract and NI-F1 on L929 cell proliferation was evaluated by scratch model and the cell viability by trypan blue. The efferocytosis activity also was evaluated *in vitro*. **Results**: In the carrageenan and LPS-induced inflammation in subcutaneous tissues was observed a markedly reduction in leukocytes migration, especially neutrophils, in animals pre-treated with *R. imperialis* extract or NI-F1. *In vitro* treatment with *R. imperialis* extract and NI-F1 promoted a decreased in NO_2 production by neutrophil stimulated by LPS. The extract and NI-F1 induced an increase in the phagocytosis of neutrophil apoptotic cells, showing a pro-resolution activity. Still, the extract and NI-F1 did not affect the cellular viability of L929 cells, and induced a proliferation of L929 cells in the scratch model in the doses of 1 and 10 µg/mL. **Conclusions**: Together, the results herein obtained provide evidences that *R. imperialis* methanolic extract and NI-F1 presents important *in vivo* and *in vitro* anti-inflammatory and pro-resolution activities. **Financial support information**: UNIVALI; CNPq (444682/2014-7).

04.052 Effect of myrtenol on neutrophil migration and adhesion in inflammatory conditions. Gomes BS[1], Sousa-Neto BP[1], Silva FV[1], Sousa DP[2], Wanderley CWS[3], Wong DVT[3], Ribeiro RA[3], Lima-Júnior RCP[3], Oliveira RCM[1], Oliveira FA[1] [1]UFPI – Medicinal Plants, [2]UFS – Pharmacy, [3]UFC – Physiology and Pharmacology

Introduction: Myrtenol is a monoterpene of pleasant smell, it is employed in the cosmetics industry, and it is part of the chemical composition of essential oils from aromatic species that presents anti-inflammatory activity. This work aims to test, evaluate myrtenol anti-inflammatory action in models of neutrophil chemotaxis *in vitro* induced by formyl-methyl-leucyl-phenylalanine (fMLP) and carrageenan-induced peritonitis analyzed by the technique of intravital microscopy. **Methods**: The chemotaxis assay was performed in 48-well microchamber (Boyden Chamber). Myrtenol (10, 30, 100 ng / well, diluted in sterile saline) or fMLP (100 ng / well) as negative control or RPMI as a positive control were added to the bottom of the wells. In the upper wells 5x10 ^{4} human neutrophils were added and the chamber was incubated for 1 h at 37° C and 5% CO $_{2.}$ The number of cells counted in five random fields per well was determined using a light microscope in triplicate. Results are expressed as number of neutrophils per well. Subsequently, evaluated the neutrophil migration in real-time in mesenteric microcirculation in mice. Initially, mice Swiss mice of 18-25g were treated orally with saline, myrtenol 25 mg/kg or dexamethasone (1 mg/kg, i.p.). One hour later, was injected carrageenan (500 µg/cavity). After 4 h, mice were anesthetized with ketamine and xylazine and mesenteric tissue was exposed for microscopic examination *in situ* by a longitudinal incision of the abdominal skin and muscle of the right side of the body, followed by the mesenteric exposure. Cells were counted in the image recorded in five different fields for each animal, in order to avoid sampling variability. The data were expressed as mean±SEM for each animal. The collection of neutrophils in humans (healthy volunteers) was approved by the report CEP. 12664/2006. The experimental protocols were approved by the Ethics Committee for Experimentation with animals of Federal University of Piauí (EAEC / UFPI, Opinion No. 008/12). **Results and Discussion**: Incubation of neutrophils with myrtenol (30 and 100 ng/mL) significantly inhibited the fMLP-induced chemoattraction (3,83 ± 0,60 e 4,20 ± 1,46, respectively), compared to vehicle group (14,00 ± 0,57). The myrtenol (25 mg/kg, po) produced a reduction in the number of rolling and adherent leukocytes (7,44 ± 1,89 e 0,20 ± 0,05, respectively). The intraperitoneal injection of 1% carrageenan (500 ug / well) in mice produced a significant increase in the number of rolling and adherent leukocytes in the endothelium (13.96 ± 3.37 and 1.60 ± 0.59, respectively). The data suggest that the myrtenol action occurs by inhibition of cell migration. However, more studies should be performed to clarify the action mechanisms of this monoterpene in the inflammatory process. **Financial support**: UFPI/CAPES.

04.053 Vascular changes and acute inflammation induced by agar in an air pouch model. Gomes MF, Avila PES, Bastos GNT, Nascimento JLM ICB-UFPA

Alternative protocols to investigate new anti-inflammatory drugs optimized for the air pouch model in rats are important pharmacological tools. Therefore, this study was designed to examine agar, as a phlogistic agent. The animals were divided into groups, in which 1%, 2%, 3% and 4% agar were used, compared to 1% carrageenan; the vehicle group was treated with saline (0.9%). For quantifying the vascular inflammation induced by agar, the microvasculature of the air pouch membrane was analyzed by adapting the NeuronJ software package. In addition, the NO-dependent pathway and cellular migration were also analyzed. The responses of the vehicle group were compared to those of groups with an air pouch inflamed induced by the injection of 2% agar or 1% carrageenan. Enhanced vasodilation responses to 2% agar or 1% carrageenan were found when compared to the vehicle group, as well as the increased production of nitric oxide and cell migration. In order to assess the mechanism of action of the phlogistic activity of agar, animals were treated with anti-inflammatory drugs; the animals were divided into groups: 2% agar, 2% agar and Celecoxib (200mg/kg), and 2% agar and ASA (100mg/kg).We analyzed the microvasculature of the air pouch membrane, performed a differential cell count from the inflammatory, nitrergic activity and cellular migration. Treatment with anti-inflammatory inhibited the activity of agar, decreasing the vasodilation, cellular migration and nitrergic activity. Thus, this study showed that a simple and alternative method produces consistent results and can be used as an alternative experimental inflammatory model. **Keywords**: Agar, Inflammation, Experimental Model, Air pouch, Wistar Rats. **Financial Support**: Capes, Fadesp **Ethics Commite**: **CEPAE - 123-13**

04.054 Evaluation of the anti-inflammatory activity of the hidroethanolic extract of *Macrosiphonia longiflora* (Desf.) Mull. Arg. in chronic pulmonar allergic inflammation experimental model. Cruz TCD, Almeida DAT, Martins DTO
Farmacologia e Toxicologia de Produtos Naturais

Introduction: *Macrosiphonia longiflora* (Apocynaceae), known as “velame-branco”, has a xilopodium used in the formo of decoccion, macerate and infusion, especially for the treatmente of chronic inflammations. **Objective**: To evaluate the activity and the mechanism of action of *EHMl* in murine modelo f chronic asthma. **Methods**: EH*Ml* was obtained by maceration of de powdered xilopodium in ethanol 70%, filtering, concentration and liofilization. For immediate hipersensibility induction in the airways, we utilized the methods of NADER et al. (2012) with adaptations. On the 1^{st} and 10^{th} days of the assay, the Swiss mice (n=6/grupo) were made sensitive with i.p. injection (200 µL) of a suspension of chicken egg albumine (OVA, degree V; 100 µg/mL) and aluminum hidroxide (10 mg/mL) in saline solution 0,9%. Between the 19^{th} and the 24^{th} days, the animals were challenged through nebulization of OVA degree II (3% in saline 0,9%),for 20 minutes per day in a closed chambre.The sensibilized and challenged with OVA animals received orally, twice a day, the solvent (Tween 20 at 2%), EH*Ml* (20, 50 and 200 mg/Kg) and dexamethasone (0,5 mg/kg). The Sham group was sensibilized e challenged with saline 0,9% and treated with the solvente alone. The bronchoalveolar (BAL) was collected for differential count in cells and the lungs harvested for histopathological examination. **Results**: The sensibilization and challenge with OVA cause increase($p<0,001$)on the BAL of 8xthe number of total leucocytes and neutrophils, 129x the number of eosinophils and 6x the number of mononuclear cells, when compared to the Sham Group ($11,77 \pm 2,14 \times 10^6$, $6,24 \pm 1,10 \times 10^6$, $0,17 \pm 0,04 \times 10^6$and $2,34 \pm 0,41 \times 10^6$, respectivelly).The treatment withEH*Ml* (20, 50 and 200 mg/kg) reduced ($p< 0,001$), when compared to the vehicle group, in all dosages and in a dose-dependent manner, the number of total leucocytes, Neutrophils, Eosinophilsand mononuclear cells, reaching, respectively, with the highest dose, the peak of the inhibitory effect(72%, 75%, 75% and 60%).Dexamethasone (0,5 mg/kg) caused inhibition ($p< 0,001$) of 86,08%on total leucocytes, 86,71% in neutrophils, 86,80% on eosinophilsand 79,08%on mononuclear cells.The histopathological analisys of the lungs demonstrated that sensibilization and challeng with OVA promoted significative increase ($p < 0,01$), in about 4 xtheoedema and the peribronchial and perivascular infiltrates, when compared to the Sham group. (1,0 - 2,0; 1,0 - 1,2 and 1,0–2,0, respectively). The treatment with EH*Ml,* reduced these three parameters only in it’s highest dose (200 mg/kg), obtaining 75% reduction of the oedema ($p< 0,01$), 75% of the bronchial infiltrate ($p<0,05$) and 75% of the perivascular infiltrate ($p< 0,05$). Dexamethasone (0,5 mg/kg), promoted reduction equivalent to that of EH*Ml*,being 75% for the o edema ($p< 0,01$), 75% for the bronchial infiltrate ($p< 0,001$) and 75% for the perivascular infiltrate ($p<0,05$). **Conclusions**: The EH*Ml* presented chronic antiinflammatory activity equivalent to that of the standard drug dexamethasone.**Financial Support**: CNPq, FAPEMAT. The assay using animals was previously approved by CEUA/UFMT, under the registration nº 23108.028369/12-4.

04.055 Effect of hydroethanolic extract of the xylopodium of *Mandevilla longiflora* **(Desf.) Pichon on the release of inflammatory mediators in murine macrophages stimulated.** Almeida DAT, Cruz TCD, Rosa SIG, Martins DTO UFMT – Ciências Básicas em Saúde

Introduction: *Mandevilla longiflora* (Desf.) Pichon (Apocynaceae), known as ”velame" is a subshrub native of Brazil and whose leaves, rhizomes and whole plant are widely used in traditional medicine in the form of decoction or infusion to the treatment of inflammations. **Objectives**: Evaluate the effect of the extract of hydroethanolic *Mandevilla longiflora* (HE*Ml*) on the release of inflammatory mediators in murine macrophages stimulated. **Method**: The 70% HE*Ml* was prepared by maceration of the powder from xylopodium (ethanol-water, filtrate and concentrate by rotary evaporation). Briefly, the viability of the RAW 264.7 cells (murine macrophages) was evaluated by submitting them to different concentrations of HE*Ml* (200 - 3.12 mg / mL) using the Alamar blue assay. HE*Ml* effect (10, 30, and 100 µg / ml) on the level of cytokines (TNF-α, IL-1β and IL-10) and PGE_2 in the supernatant was analyzed after stimulation of cells with LPS (4 or 24 h), using an ELISA kit. Also, the NO production was assessed after pre-treatment with HE*Ml* and stimulus with LPS (1 µg / ml) + IFN-γ (1 ng / ml) of the RAW 264.7 cells, using colorimetric method of Griess. **Results/Discussion**: RAW 264.7 cells viability was not affected by HE*Ml* (IC50> 200 mg / mL), indicating that the extract have non-cytotoxic effect. The TNF-α, IL-1β, IL-10 and PGE_2 levels of the RAW 264.7 cells after LPS-stimulated were 3.31 ± 0.08, 20.42 ± 1.88, 303.70 ± 2.41 and 12.46 ± 1233.00 pg / ml, respectively. Pretreatment with HE*Ml* reduced levels of these four mediators, whose effective concentrations and intensities of the responses varied according to the mediator, reaching the highest inhibitory effects with 100 µg / mL for PGE_2 (187.50 ± 27.01 pg / mL, $p < 0001$) and IL-1β (6.08 ± 1.52 pg / ml**,** $p < 0.01$). Dexamethasone (10 mM), standard anti-inflammatory drug, caused reduction in TNF-α levels by 14.8% ($p < 0.001$), IL-1β in 80.0% ($p < 0.001$) and IL-10 in 25.4% ($p < 0.05$). NS-398 (10 mM), COX-2 inhibitor, reduced the PGE_2 concentration in 85.2% ($p < 0.001$). The concentration of NO in LPS + IFN-γ -stimulated RAW 264.7 was 33.16 ± 1.9 µM. Pretreatment with HE*Ml* reduced concentration-dependently the levels of NO, reaching the peak effect 100 µg/ ml (7.24 ± 1.25 µM, $p < 0.001$), while L-NAME (10 mM) reduction was 80.4% ($p < 0.001$). **Conclusion**: The anti-inflammatory activity of HE*Ml* attributable to its action on multiple targets inflammatory process. **Acknowledgement**: CNPq, CAPES, FAPEMAT, INAU Keywords: *Mandevilla longiflora*, anti-inflammatory, RAW 264.7

04.056 Role of tumor necrosis factor-alpha on platelet reactivity of rats injected with lipopolysaccharide. Bueno PI, Abreu E, Naime ACA, Bonfitto PHL, Goulart G, Marcondes S FCM-Unicamp – Farmacologia, [2]Unicamp – Farmacologia

Introduction: Lipopolysaccharide (LPS), a main constituent of Gram-negative bacterial membrane, is largely used as a tool to study sepsis, a problem in all over the world. Platelets have been described as important cells in sepsis, and the reduction of circulating platelets is considered a marker of the severity of this condition. Recently, our group showed that the treatment of rats with lipopolysaccharide (LPS) causes thrombocytopenia, reduces aggregation and increases reactive oxygen species (ROS) formation in platelets, but the last two effects is not observed when platelets are incubated with LPS. **Objective**: The objective of the present work was investigate if tumor necrosis factor-alpha (TNF-α), a cytokine largely produced in sepsis, modulates the thrombocytopenia and the platelet reactivity in LPS-injected mice. **Methods**: The present study was approved by the institutional Committee for Ethics in Animal Research/State University of Campinas (protocol 3715-1, 3290-1, 3533-1). We treated the mice with the antibody anti-TNF-α (infliximab, 10 mg/kg, sc) 30 min before LPS injection and at 48h thereafter arterial blood was collected. We evaluated the counts of platelets in peripheral blood, aggregation and ROS formation in platelets. ROS levels were determinated by flow cytometry. **Results**: Infliximab did not prevent the thrombocytopenia after LPS injection, but significantly augmented the platelet aggregation compared to the group injected just with LPS (increase of 52%). In ADP-activated platelets of LPS-injected mice, generation of ROS was increased by 2.9-fold compared to the saline-injected group. This increased ROS production was totally prevented when the mice were treated with infliximab before LPS injection. Aggregation or ROS generation in platelets were not affected in the group injected only with infliximab compared to the group injected with saline. **Conclusion**: TNF-α does not participate of the thrombocytopenia observed in the sepsis induced by LPS. However, TNF-α has an important role in the inhibition of aggregation and in the increased ROS generation in platelets in this experimental model of sepsis.

04.057 Mechanisms involved in the peripheral anti-inflammatory effect of tramadol into rat's temporomandibular joint. Lamana SMS, Nascimento APC, Napimoga MH, Araújo DR, Furtado FF, Macedo CG, Clemente-Napimoga JT FOP-Unicamp – Ciências Fisiológicas

Introduction: Tramadol hydrochloride is a centrally acting analgesic drug used mainly for the treatment of moderate to severe, as well as acute and chronic pain, however there are not enough studies supporting the peripheral anti-inflammatory effect of tramadol. **Aim**: The aim of this study was evaluate the peripheral anti-inflammatory effect of tramadol through the induced inflammatory pain in the rats TMJ. **Methods**: Wistar male rats (±150 g, n=4/group) were treated with an intra-TMJ injection of Tramadol (0.025, 0.25, 0.5 or 1 mg/TMJ) co-administrated with 1.5% Formalin and non-selective antagonist of opioid receptors (Naloxone) or selective inhibitor of PI3K (AS605240); selective inhibitor of AKT (AKT inhibitor IV) or inhibitor of nitric-oxide synthase (L-NMMA); inhibitor of soluble cGMP enzyme (ODQ) or inhibitor of PKG (KT5823) or ATP-potassium sensitive channel blocker (Glibenclamide). Besides, animals were pretreated (15min) with an intra-TMJ injection of Tramadol (0.125, 0.25 and 0.5µg/TMJ) followed by: (1) an ipsilateral intra-TMJ injection of carrageenan (100µg/TMJ) 1 hour prior an intra-TMJ injection of a low dose of 5-hidroxytryptamine (5-HT, 75µg/TMJ) or (2) an intra-TMJ injection of 5-HT (225µg/TMJ). Animals' nociceptive behavior was observed during 45-30 minutes and then they were terminally anesthetized and their periarticular tissue removed for leukocytes migration, plasma extravasation, western blotting analysis and inflammatory cytokines release measurements. **Results**: Intra-TMJ injection of Tramadol 0.25, 0.5 and 1 but not 0.025 mg/TMJ significantly reduced nociceptive behavioral responses induced by formalin ($p<0.05$: ANOVA, Tukey's test). Pretreatment with L-NMMA and ODQ but not Naloxone, AS605240, AKT, KT5823 and Glibenclamide significantly reduced the peripheral antinociceptive effect induced by intra-TMJ injection of Tramadol (0.5 mg/TMJ) ($p<0.05$: ANOVA, Tukey's test). Pretreatment with Tramadol 0.5 but not 0.25 and 0.125 µg/TMJ significantly reduced nociceptive behavioral responses induced by carrageenan or 5-HT ($p<0.05$: ANOVA, Tukey's test). The pretreatment with Tramadol (0.5 mg/TMJ) did not affect the expression of ICAM and CD55 ($p>0.05$: ANOVA, Tukey test) but significantly reduced the release of TNF-α and IL-1β induced by formalin and carrageenan ($p<0.05$: ANOVA, Tukey's test). The pretreatment with Tramadol (0.125, 0.25 and 0.5 µg/TMJ) significantly reduced leukocytes migration and plasma extravasation ($p<0.05$: ANOVA, Tukey test). **Conclusion**: The results suggest that Tramadol induces an anti-inflammatory effect mediated by the activation of intracellular NO/cGMP pathway and the inhibition of hypernociceptive inflammatory cascade. **Research support**: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP #2013/11090-0), Brazil. Committee on Animal Research at the UNICAMP (#2983-1).

04.058 *Porphyromonas gingivalis* **lipopolysaccharide increases the expression and activity of metalloproteinase-9 in gingival fibroblasts culture from normal and diabetic mice.** Beltran CT, Tirado IS, Brito VGB, Queiroz DPS, Oliveira SHP Unesp-Araçatuba

Introduction: Periodontal disease, a grievous inflammatory condition affect all periodontal structures. It can have more severe consequences when developed in diabetic patients with poor glycemic control. Matrix metalloproteinases (MMPs) are important extracellular components that participate on cell matrix remodeling in the physiological process. MMPs also have been reported to play an important role on pathological conditions presenting tissue destruction, as in the periodontal disease. **Aims**: To evaluate the expression and activity of MMP-9 by gingival fibroblasts from diabetic mice stimulated with Porphyromonas gingivalis lipopolysaccharide (PG-LPS). **Methods**: Males Balb/c mice were divided in two groups (normal and diabetic). Experimental protocol was approved by Ethics Committee on Animal Experimentation from School of Dentistry of Araçatuba (Process 01342/2014). Diabetes induction was conducted by streptozotocin intraperitoneal administration (200 mg/Kg), at 7 days later, blood glucose concentration was measured and mice presenting values over than 250 mg/dL were considered diabetics. Normal and diabetic animals were euthanized; gingival tissue was surgically collected and used for primary gingival fibroblasts culture (pGFC) establishment. Culture flasks were maintained in culture medium (Dulbecco's Modified Essential Medium plus 10% fetal bovine serum) in 37°C humidified atmosphere with 5% CO_2. Experiments were conducted with cultures between 4 to 5 passages, in 24-well plates with 100000 cells/mL density seeding. PG-LPS at different concentrations (0,01; 0,1; 1 and 10 µg/mL) were used to stimulate pGFC. Cell viability, MMP-9 mRNA expression and activity were evaluated by MTT assay, real time RT-PCR and gel zymography, respectively. **Results**: Initially, PG-LPS had no effect on cell viability on normal and diabetic groups, however at 24 hours MTT assay showed 4 to 8,5% viability decrease in the normal group. On the other hand, diabetic group demonstrated a slight metabolic increase, at the same period. Real time RT-PCR showed a significant increase in MMP-9 mRNA level after 1 and 10 µg/mL at all period analyzed, with accentuated pick at 6 hours observed in normal and diabetic groups.The MMP-9 enzyme activity picked was at 24 hours observed in normal and diabetic group, however, the sensibility of the response was lower in the diabetic group, since the concentration used to stimulate the diabetic cells was 0,01 µg/mL. **Conclusion**: PG-LPS was able to increase MMP-9 expression and enzyme activity, further studies are been conducted by our group to elucidate the mechanism and intracellular signaling pathway involved in the inflammatory scenery. **Financial support**: CAPES and FAPESP

04.059 Topical formulation containing microencapsulated rutin reduces UVB irradiation-induced skin oxidative stress and inflammation. Medeiros DC[1], Martinez RM[2], Mizokami SS[3], Pinho-Ribeiro FA[3], Georgetti SR[2], Baracat MM[2], Verri Jr WA[3], Casagrande R[2] [1]UEM – Ciências Farmacêuticas, [2]UEL – Ciências Farmacêuticas, [3]UEL – Ciências Patólogicas

Introduction: Many novel delivery systems have been designed for topical application of drugs since they can overcome the stratum corneum barrier and increase drug permeability, improving the prevention of ultraviolet B (UVB) irradiation-induced skin inflammation. In addition to novel delivery systems, natural products have been used as novel anti-inflammatories. For instance, rutin is a flavonoid with significant antioxidant and anti-inflammatory activity. **Aims**: The present study developed a topical formulation containing microencapsulated rutin and determined its efficacy against UVB-induced skin inflammation and oxidative stress in hairless mice. **Methods**: Skin inflammation and oxidative stress were induced by UVB irradiation. Skin edema was evaluated by the weight change, matrix metalloproteinase-9 activity by zymography, oxidative stress was evaluated by measuring of ferric reducing antioxidant power (FRAP), ability to scavenge the radical ABTS, reduced glutathione levels, and catalase activity. It was also investigated the NADPH oxidase sub-unit gp91phox and cytokines mRNA expression by qPCR. **Results**: Topical treatment with microencapsulated rutin prevented UVB-induced skin inflammation by reducing skin edema (100%) and matrix metalloproteinase-9 activity (71.65%). Topical formulation containing microencapsulated rutin also inhibited UVB-induced gp91phox and cytokines (TNF-α, IL-1β, and IL-10) mRNA expression and oxidative stress (FRAP: 71.04%; ABTS: 35.0%; GSH: 69.0%, and catalase: 74%). These effects were not observed with the application of topical formulation containing non-microencapsulated rutin or blank microcapsules. **Conclusions**: Microencapsulation of rutin resulted in enhanced topical antioxidant and anti-inflammatory efficacy compared to non-microencapsulated rutin and blank microcapsules. Therefore, the release modification of the natural molecule rutin improved its efficacy indicating that modifying drug release is a conceivable approach to a better use of natural resources. **Financial support**: CNPq, CAPES, MCTI, SETI, Fundação Araucária and Paraná State Government.

Experimental procedures and animal care were approved by CEUA-UEL under the process number 27025.2013.10.

04.060 Physicochemical characterization of 15d-Prostaglandin J2-loaded solid lipid nanoparticles and effects on inflammation. de Melo NFS[1], Macedo CG[2], Abdalla HB[2], Bonfante R[2], Fraceto LF[3], Clemente-Napimoga JT[2], Napimoga MH[1] [1]São Leopoldo Mandic – Imunologia e Biologia Molecular, [2]FOP-Unicamp – Fisiologia, [3]Unesp – Engenharia Ambiental

Introduction: 15-deoxy-delta^{12,14}-prostaglandin J_2 (15d-PGJ_2) is a PPAR-gamma endogenous ligand with physiological properties including potent anti-inflammatory activity, although much of exogenously administered 15d- PGJ_2 binds to serum albumin. So, an alternative that has been able to avoid this problem is modified release through the solid lipid nanoparticles (SLN).The SLN can improve the therapeutic properties of the drug incorporated, such as increased drug stability and prolonged release. **Aim**: The objective of this work was to develop SLN formulation for modified release of 15d-PGJ_2 as well as conduct the investigation of immunomodulatory potential of the suspension, aiming a new system for future use in the treatment of inflammation. **Methods**: The SLN suspension containing 15d-PGJ_2 were prepared using the emulsification/solvent evaporation method and were characterized (size, polydispersivity, zeta potential, pH). The stability was investigated as a function of time. The 15d-PGJ_2 release profile was determinated using two compartments model. Mice were pretreated with 15d-PGJ_2: SLN (3, 10 or 30 $\mu g \cdot kg^{-1}$), before induction of an inflammatory response by intraperitoneal injection of carrageenan or LPS. Total counts were performed in a Newbauer chamber, and differential cell counts (100 cells total) were carried out on cytocentrifuge. **Results**: The 15d-PGJ_2: SLN presented diameter around 260 nm, polydispersity below 0.2 indicating good homogeneity of the formulations, zeta potential lower than -25 mV and 15d-PGJ_2 loading up to 90%. The results showed that the formulation was stable up to 120 days. 15d-PGJ_2 release profile was modified by nanoparticles. Neutrophil migration induced by administration of carrageenan and LPS was inhibited by 15d-PGJ_2-NC, but not by unloaded 15d-PGJ_2. The results indicate the potential of the novel anti-inflammatory 15d-PGJ_2: SLN formulation. **Conclusion**: The new 15d-PGJ_2: SLN formulation presented good colloidal properties, modified release and enables the use of a smaller drug dose and is significantly effective. **Acknowledgements**: FAPESP and CNPq. Animal Research Ethical Committee: CEUA Unicamp 3623-1.

04.061 Extracellular adenosine orchestrates sepsis-induced immunossupression through activation of A2a receptor. Nascimento DC, Melo PH, Ferreira RG, Peres RS, Cunha FQ, Alves-Filho JC FMRP-USP – Farmacologia

Introduction: Sepsis leads to a long-term immunosuppression state that is characterized by M2-like macrophage polarization and expansion of regulatory T cells (Treg). However, the molecular mechanism(s) underlying the expansion of Tregs after sepsis remain unclear. Adenosine, a purine nucleoside, has been shown to increase the numbers of Tregs through activation of A2a receptor (A2aR) and further promotes their immunoregulatory activity. In this study, we investigate the role of ectonucleotidases CD39 and CD73, the main pathway for extracellular adenosine production, and A2aR in the establishment of sepsis-induced immunosuppression. **Methods and Results**: All experiments were approved by the local ethics committee (Protocol number: 176/2011). Mice were subjected to severe sepsis by cecal ligation and puncture (CLP) model and treated with antibiotic (ertapenem), resulting in 40% of survival. At 15th day after CLP, sepsis-surviving mice showed an increased number of Tregs (CD4+Foxp3+ T cells) in spleen and impaired T-cell proliferative response, which was associated with high susceptibility to a secondary infection induced by *Legionella pneumophila*. Notably, deficiency of A2aR or treatment with A2aR antagonist [8-(3-chloro-styryl)caffeine] reduced the number of Tregs and improved proliferative response by T cell in sepsis-surviving mice. Moreover, sepsis-surviving mice treated with CD39 inhibitor [ARL671516] or CD73 inhibitor [adenosine 5′-(α,β-methylene)diphosphate] also showed reduced number of Tregs and improved T-cell proliferative response. In accordance, deficiency of A2aR or pharmacological inhibition of A2aR, CD39 or CD73 improved bacterial clearance and rendered sepsis-surviving mice more resistant to a secondary challenge with *L. pneumophila* infection. Finally, we found that macrophages from sepsis-surviving mice, mainly M2-like macrophages, show an increased expression of CD39 and CD73, suggesting that macrophages may represent an important source of adenosine after sepsis. **Conclusion**: our results suggest that extracellular production of adenosine by CD39/CD73-expressing macrophages establishes an immunosuppressive microenvironment that promotes of Tregs expansion and mediates immunosuppression in sepsis-surviving mice. **Financial support**: FAPESP.

04.062 Role of intestinal microflora and bacterial translocation in the pathogenesis of steatohepatitis induced by irinotecan in mice. Aragão KS[1], Almeida PRC[2], Melo AT[1], Muniz HA[3], Lopes CDH[3], Neto PRP[3], Carvalho CBM[4], Lima-Júnior RCP[1], Ribeiro RA[1] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Patologia e Medicina Legal, [3]Hospital Haroldo Juaçaba/ICC, [4]UFC – Medical Microbiology

Introduction: Nonalcoholic steatohepatitis (NASH) is a new complication of irinotecan (IRI)-based anticancer regimens. NASH may worsen the clinical course of patients submitted to hepatic metastasis resection of color rectal cancer. Recently, we developed a new experimental animal model of the IRI-induced steatohepatitis with the full histological findings seen in the human NASH. We aimed to investigate the role of intestinal microflora and bacterial translocation in IRI-induced NASH pathogenesis. **Methods**: We used an antibiotics association (ANTB) to sterilize the intestinal mice microbiota for studying the role of bacteria translocation in NASH. C57BL/6 mice(n=8,25g) were divided into groups and injected with saline (5ml/kg,ip). We put a cocktail of ANTB in the drinking water (7 weeks) (all at 50mg/kg body weight/day). Fecal samples were cultured using a BHI test after 10 days of ANTB. On day 10, we submitted animals to IRI treatment (50mg/kg,ip,3x/week/7weeks). After 7 weeks, peripheral blood was collected for measuring serum enzyme ALT(U/L) and bacteremia. Animal livers were used for histopathological analysis by the Kleiner scores (lobular inflammation[0-3], steatosis[0-3] and vacuolization[0-3]), IL-1β and TNF-α cytokines analysis, and also for immunohistochemical(IHC) assay. We performed intestinal histopathologic analysis. Data analysis presented $p<0.05$ through ANOVA/Student Newman Keul or Kruskal Wallis/Dunn tests. **Results**: IRI induced a marked decrease in body weight in mice (36%), an increase in the serum concentrations of ALT (25.4±3.2 *vs* control group 17.3±5.2), and a liver wet weight (1.962±165.3 *vs* 1.425±39.5). It also induced local production of IL-1β (977.8± 55.6 *vs* 624.9±49) and TNF-α (1775±76.7 *vs* 1434.8±187.6), and an increase in Kleiner's scores 5(3–7) *vs* 2(1–3). Bacteremia was evidenced in IRI-injected group (portal blood-80%) and (systemic blood-40%) versus saline group(0%). Shortening of the villi and the alteration of crypt size and architecture were also observed in IRI group (12[8-17] *vs* 2,5[2-3]). ANTB maintains intestinal bacteria depletion for over than 7 weeks during IRI-induced NASH. Interestingly, ANTB prevented the decrease of body weight induced by IRI ($p<0.05$). In addition, ANTB prevented the increase of ALT(15.8±1.5), liver wet weight(1,366±74.3), inhibited tissue production of IL-1(748.2±28.9) and TNF-α(1565±27.7), and prevented the increase in Kleiner's scores(3[1–7]) *vs.* the IRI group. In addition, antibiotic therapy abolished irinotecan-related bacteremia(0%) versus IRI group. IHC analysis in liver samples of the IRI group showed a significant increase for iNOS(3[2–3] *vs* 2[1–2]) and TLR4(2[0-3] *vs* 0[0–1]) immunostaining, which was prevented by ANTB (iNOS: 1,5[1–3]; TLR4: 0[0-2]). IHC analysis in intestinal samples of IRI-injected group showed a significant increase in TLR4 (1[0–2] *vs* 0[0–0]) which was prevented by ANTB (TLR4: 0[0-1]). **Conclusion**: These results suggest IRI causes rupture of gut barrier. The intestinal bacterial translocation from the intestine seems to be a key factor leading to the progress of IRI-induced NASH. **Support**: CNPq. **Animal research ethical committee**: 21/2012.