04. Inflammation

04.001 Evaluation of the involvement of endothelins in the inflammatory process induced by superoxide anion. Serafim KGG, Zarpelon AC, Verri Jr WA UEL – Ciências Patológicas

Introduction: During the inflammatory process NADPH oxidase is activated and it produces superoxide anion, which in turn, induces the expression of proinflammatory molecules related to the nuclear transcription factor NF-κB. The inflammatory mediators produced by superoxide anion in other systems include endothelin-1 (ET-1). Furthermore, ET-1 induces the production of superoxide anion by NADPH oxidase, indicating a possible feedback relationship between superoxide anion and ET-1 in inflammation. As a consequence of the above mentioned there is inflammatory hyperalgesia, edema and neutrophils recruitment. In the present study it was investigated in vivo whether superoxide anion-induced inflammation depends on ET-1. **Methods**: Male Swiss mice, 20-25g from Universidade Estadual de Londrina were used in this study with the approval of the local Ethics Committee (no. 07882). Mice were treated with bosentan (mixed ET_A and ET_B endothelin receptor antagonist, 10, 30 and 100 mg/kg , per oral) 1 h before the intraplantar stimulus with KO_2 (superoxide anion donor, 30 µg/paw). Mechanical (electronic version of von Frey filaments) and thermal (hot plate) hyperalgesia and edema (analog caliper) were evaluated between 0.5-7h, and at 7 h animals were killed and paw skin samples analyzed for myeloperoxidase activity (colorimetric assay). In overt pain-like behavior (paw flinching and licking) analysis, mice were treated with bosentan (100 mg/kg , per oral) 1 h before KO_2 (30µg/paw) injection. Abdominal contortions were induced by KO_2 (1mg/mice, intraperitoneal injection) and evaluated in mice treated with vehicle or bosentan (100 and 300 mg/kg , per oral) 1 h before stimulus. In leukocyte recruitment to the peritoneal cavity experiments, mice were treated with bosentan (100 mg/kg , per oral) 1 h before KO_2 (30µg/mice, peritoneal cavity) injection. After 6 h mice were killed and peritoneal cavities exudates were used to determined the number of total leukocytes, mononuclear and polymorphonuclear cells. Statistical analysis were performed using Graph Pad Prism 4.0, One-way ANOVA followed by Bonferroni's t test and significant differences considered using $P<0.05$. **Results:** Bosentan inhibited in a dose-dependent manner KO_2-induced inflammation and maximal effects were achieved with the dose of 100 mg/kg , therefore, the results of this dose are described as following: bosentan a) decreased mechanical hyperalgesia after KO_2 stimuli up to 77% at 7h; b) thermal hyperalgesia was reduced up to 100% at 3 hours; c) edema was reduced up to 67% at 30 min; d) myeloperoxidase activity was reduced up to 80%; e) number of flinches were reduced in 30%; f) time spent licking the paw was reduced in 67%; g) abdominal contortions were inhibited up to 53%, and in this response 300 mg of bosentan were used, but are acceptable since a higher dose of KO_2 is necessary to induce this response; g) decreased in 65% the number of total leukocytes, 38% mononuclear cell and 73% neutrophils. **Conclusion:** Endothelin receptor antagonists might be promising as an anti-inflammatory and analgesic approach in superoxide anion mediated inflammation. Further studies are necessary to unveil the relative contribution of ET_A and ET_B endothelin receptors. **Financial support**: CNPq,CAPES, SETI/Fundação Araucária and Governo do Estado do Paraná.

04.002 Leptin upregulates lipid mediators expression in primary culture of pulmonary endothelial cells activated by LPS. Gasparin RM[1], Landgraf MA[2], Santos LA[2], Azevedo RL[2], Câmara NOS[3], Fernandes L[2], Landgraf RG[2] [1]Unifesp, [2]Unifesp – Ciências Biológicas, [3]USP – Imunologia

Introduction: The 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. Activated vascular endothelium expresses adhesion molecules and chemokines, which stimulate leukocytes recruitment and migration process to the inflammatory sites. Leptin is a hormone mainly synthesized by adipose tissue; it is involved in various biological systems, acting in the food intake control and energetic metabolism, in addition to modulate immune response, hematopoiesis and lymphopoiesis. Culture of pulmonary endothelial cells may represent important tools for understanding the interaction between these cells and leukocytes trafficking. Objectives: Standardization and characterization of primary culture of C57Bl/6 mice pulmonary endothelial cells and evaluation of production of inflammatory mediators (PGE_2, LTC_4, LTB_4) in these cells, stimulated or not with LPS and/or leptin. **Methods**: Male C57Bl/6 mice were euthanized and lung tissue samples were isolated under sterile conditions, minced, covered with supplemented DMEM (20% fetal bovine serum and 1% penicillin/streptomycin 100 U/mL) and kept in an incubator at 37°C and 5% CO_2. 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 CD34 (L-selectin), CD105 (endoglin), CD106 (V-CAM) and CD45 (marker of hematopoietic origin cells). After the characterization, these cells were stimulated or not with LPS (1 µg/mL) and/or leptin (10 ng/mL), for 6 hours to evaluate the production of inflammatory mediators such as PGE_2, LTB_4 and LTC_4/D_4. All the procedures used in this study were approved and are in accordance with the rules established by the Ethics Committee of UNIFESP (CEP-1038/11). **Results:** The cells were positive for all the markers used, except for CD45. The leptin stimulus did not alter the levels of the inflammatory mediators studied. LPS increased the PGE_2 levels (319%) and the leptin addition potentiated it by 30%. Interestingly, we found that neither LPS nor leptin are alone able to alter the leukotrienes levels, but when administered together, LPS and leptin increased the LTB_4 and LTC_4/D_4 production in 293% and 374%, respectively. Conclusion: Our preliminary results suggest that leptin plays an important pro-inflammatory role in the cultures of mice primary endothelial cells, since this hormone potentiated the LPS effect. **Financial support**: FAPESP (2010/01404-0, 2011/09947-5, 2009/52119-6), FADA-UNIFESP and CAPES.

04.003 Increased inflammatory response induced by new strain of *Proteus mirabilis* is modulated by leukotrienes expression. Santos LA[1], Ferreira RR[2], Gasparin RM[1], Tambellini VY[2], Silva RC[3], Landgraf MA[1], Landgraf RG[1] [1]Unifesp – Ciências Biológicas, [2]ICB-USP – Biotério Central, [3]Unifesp – Medicina Translacional

Introduction: *P. mirabilis* is a gram negative bacillus belonging to the family Enterobacteriaceae, described as an etiologic agent in various infections. Objectives: Evaluate the pulmonary inflammatory response in mice infected with different strains of *P. mirabilis*. **Methods**: Groups of 6-8 male C57Bl/6 mice (20-25g) were infected with a suspension containing 10^{-1} CFU of *P. mirabilis* ATCC 25933, NCDC 2059-70 *(*40 µl, i.n.) or with 10^{-1} CFU of *P. mirabilis (*40 µl, i.n.) isolated from the lungs of mice in routine screening of the Control Laboratory of the Health Central Animal ICB/USP (CAM strain). Twenty-four hours after infection, mice were given LPS (*E. coli* – Sigma, i.n.). After a further period of 24 hours, mice were euthanized with overdose of anesthetic (150 mg/kg) and the bronchoalveolar lavage fluid was collected to evaluate total and differential cellular infiltration in lung. A fragment of lung tissue was removed, fixed and stained for subsequent histological analysis and other fragment is prepared to lipid mediators quantification. All the procedures used in this study were approved and are in accordance with the rules established by the Ethics Committee of UNIFESP (CEP-1666/09). **Results:** The administration of LPS increased 48x the total cells in bronchoalveolar lavage fluid, when compared to control mice. Similar result was obtained in mice infected with ATCC (50x). The *influx* of inflammatory *cells* into bronchoalveolar lavage fluid was higher in mice infected with ATCC (60%) plus LPS than the group infected with only ATCC. The CAM strain induced significant cell infiltration (89x), in bronchoalveolar lavage fluid, when compared to animal control; the administration of LPS in mice infected with the CAM strain increased cellular infiltration in 101%, when compared to mice that were infected with CAM strain. Histological analysis of cell infiltrate in lung tissue showed that both infection with ATCC (168%) and CAM strain (225%) induced increased cell influx into the lung, when compared to control group. After LPS administration, mice infected with the CAM strain showed increased lung inflammatory infiltrate (25%) when compared with the ATCC strain. The administration of LPS increased 94% PGE2 production when compared to control group and this increase has not been altered in any of the experimental groups. LTB4 production was no different between the groups, however levels of LTC4 were increased (405%) only in groups infected with the CAM strain **Discussion/Conclusion**: Administration of LPS induced a higher lung inflammation response in mice infected with *P. mirabilis* CAM strain than in mice infected with *P. mirabilis* ATCC strain. Acknowledgements: FAPESP (2010-01404-0), Capes and FADA-UNIFESP.

04.004 Role of TRPV1 and TRPA1 receptors in skin inflammation induced by formaldehyde, **xylene and toluene in mice**. Norões MM, Cavalcante JM, Soares BL, Gavioli EC, Soares-Rachetti VP, André E UFRN – Farmacologia Comportamental

Introduction: The molecular mechanisms of contact dermatitis induced by chemical irritants are still unclear. Several studies related that irritants compounds can activate primary sensory fibers producing neurogenic inflammation and exacerbation of inflammatory responses. TRPV1 and TRPA1 receptors are expressed in primary sensory fibers and both can work as molecular integrators of noxious stimuli. Thus, the aim of this study was to investigate the role of these receptors in skin inflammation evoked by chemical irritants. **Methods**: Experiments were performed on adult male Swiss mice (weight 25–35 g). Formaldehyde (37%), Xylene (100%), Toluene (100%) or Vehicle (Acetone) was topically applied on the mouse´s ear. The ear edema was expressed as the increase in ear thickness due to the chemical irritants challenge. Separate groups of animals were topically pretreated with TRPV1 antagonist (SB366791, 200 nmol/20 ul) and TRPA1 antagonist (HC030031, 300 nmol/20 ul) 15 minutes prior to the chemical irritants application. The study was approved by UFRN's Ethics Committee on Animal Use (Protocol N°: 023/2010). **Results and Discussion**: Topical application of Formaldehyde, Xylene and Toluene induced ear edema (mean of 0.121; 0.080 and 0.067 µm, respectively, $p<0.0001$, n = 10) compared to vehicle (Acetone; mean of 0.014 µm, n = 12). The ear edema evoked by Formaldehyde, Xylene and Toluene was inhibited significantly by SB366791 (mean of 72%, 60% and 42%; respectively, $p< 0.001$, n = 10) and HC03001 (mean of 44%, 39% and 51%, respectively, $p<0.001$ n = 9). In addition, the neonatal capsaicin treatment largely reduced the edema induced by Formaldehyde, Xylene and Toluene (mean of 69%, 87%, 54%, respectively, $p<0.005$, n = 7). Our findings suggest a possible role of TRPV1 and TRPA1 on skin inflammatory responses and a possible strategy in drug development to the treatment of irritative dermatitis induced by chemical irritants. **Financial support**: CNPq.

04.005 Characterization of the anti-inflammatory effect from the essential oil of *Citrus latifolia* Tan. Amorim JL[1], Pinheiro MMG[1], Simões AC[2], Tinga ACC[3], Alviano DS[3], Silva AJR[2], Alviano CS[3], Fernandes PD[1] [1]ICB-UFRJ, [2]UFRJ – Natural Product, [3]UFRJ – Microbiology

Introduction: *Citrus latifolia* Tan. (CL), also known as "Limão tahiti", is a citric fruit of the family Rutaceae and some studies indicate their anti-inflammatory activity. The plant material has been identified by Dr. Rosana C.Lopes (Biology Institute, UFRJ) and a voucher sample was deposited in the Herbarium of the Department of Botany, Federal University of Rio de Janeiro, under number 13,150. The aim of this work was to evaluate the anti-inflammatory effect of the essential oil from the barks of CL in the model of carrageenan-induced inflammation into the subcutaneous air pouch (SAP). **Methods**: Samples of CL were purchased in popular market. The oil from CL barks was obtained by hydrodistillation in a Clevenger apparatus. The model used was carrageenan-induced inflammation into the SAP. Male mice (20-25g; n = 6-8) received oral administration of CL (10, 30 or 100 mg/kg) 1h before carrageenan injection into the SAP. After 24h the mice were euthanized and the exudates from SAP were collected to measurements of cell count, protein extravasation, nitric oxide (NO), and cytokines. The NO production was measured by the concentration of nitrate (stable metabolite) using the technique of converting nitrate followed by reaction Grees. The protocol for the use of animals received the number ICBDFBC015. Statistical significance between groups was determined by ANOVA followed by Bonferroni´s test (*$p<0.05$). **Results:** CL showed significant anti-inflammatory activity in all doses tested with reduction on cell migration (control = 58.6±6.8x10^6cells/mL *versus* 40.7±5.4*x10^6 cells/mL, 22.6±7.1*x10^6cells/mL and 11±4.6*x10^6cells/mL) and protein extravased (control = 213.5±33.4mg/mL *versus* 143.1±15.3*mg/mL, 84.8±12.6*mg/mL and 69.6±11.5*mg/mL) with the doses of 10, 30 and 100 mg/kg , respectively. Pre-treatment with CL also significantly and dose-dependently inhibited NO production (control = 207.1±31.8µM *versus* 174.9±21.1µM, 77.6±11*µM and 65.2±18.8*µM to 10, 30 and 100 mg/kg , respectively). The following cytokines also had a decrease in the treated groups: TNF-α (control = 674.1±66.1pg/mL *versus* 630.4±55.9pg/mL, 394.4±81.6*pg/mL, 386.2±45.8*pg/mL), IL-1β (control = 1,320±8.4pg/mL *versus* 1,252±133.1pg/mL, 827.3±68.7pg/mL and 874.1±92.4pg/mL) and INF-γ (control = 914.8±66.6pg/mL *versus* 488.8±45.4*pg/mL, 27.5±13*pg/mL and 18.4±13.8* pg/mL) to 10, 30 and 100 mg/kg , respectively. **Discussion**: The results demonstrate that the essential oil of CL produces anti-inflammatory effect by reducing cell migration, protein extravasation, NO, and cytokines production. A possible explanation for these effects could be the inhibition of TNF-α and INF-γ, knowing these cytokines to be involved in acquired cellular immunity and acting synergistically to increase the expression of MHC class I in many cell types. The MHC class I molecules are present on the surface of all nucleated cells and present antigens to CD8 lymphocytes. The differentiation of CD4 in T helper type 1 (Th1) occurs in the presence of IFN-γ which will determine an inflammatory profile. CL also inhibited the production of NO and their involvement in the acute inflammatory response may be related to its ability to increase vascular permeability and edema through changes in local blood flow and increased production of pro-inflammatory prostaglandins. The mechanism(s) by which the oil produces this effect are still under investigation. **Financial support**: CAPES, CNPq, FAPERJ, and IVB.

04.006 Adenosine deaminase activity as a biochemical marker of inflammatory response in goats infected by caprine arthritis-encephalitis virus. Cavalcante IJM[1], Rodrigues LFS[2], Vale MR[1], Nunes MO[1] [1]UFC – Physiology and Pharmacology, [2]UFRA – Animal Health and Production

Introduction: Caprine arthritis-encephalitis (CAE) is a form of chronic and degenerative arthritis caused by a retrovirus and is associated with important economic losses. Adenosine deaminase (ADA) levels increase in some human infectious diseases, such as tuberculosis and acquired immune deficiency syndrome. Due to the similarity between CAE virus (CAEV) and human immunodeficiency virus, we hypothesized that ADA activity in goats with clinical signs of CAEV infection is also altered and could serve as a valuable biochemical marker. **Methods**: Adenosine deaminase was assayed using adenosine (Ado) or 2'-deoxyadenosine (dAdo) as substrates, and ADA activity was calculated using the amount of ammonium produced. The experimental protocols (# 65/2011) were approved by the Institutional Committee on the Care and Use of Animals for Experimentation of Federal University of Ceará, in accordance with the guidelines of the Brazilian Committee of Animal Experimentation (COBEA). **Results:** No significant difference was detected in the activity of the serum enzyme when assayed with Ado (Km = 49.19 ± 5.28 µmol/L) or dAdo (km = 41.28 ± 4.58 µmol/L). Caprine serum ADA is a thermo-stable enzyme and can be stored at cool temperatures for at least 30 days with no loss of activity. **Discussion**: An increase in ADA activity (approximately 2.6-fold) was found in serum and synovial fluid in animals with clinical signs of CAE compared with animals without these clinical signs. In serum, the ADA cutoff value for CAE using Ado was > 34.9 U/L. Adenosine deaminase activity may be used as an important biochemical marker of the inflammatory response induced by CAEV, and its determination in serum using adenosine as a simple and inexpensive method is sufficient to assess the pathological condition of the animal.

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04.007 Pyrrolidine dithiocarbamate inhibits UVB-induced skin oxidative stress and inflammation in hairless mice. Ivan ALM[1], Campanini MZ[1], Martinez RM[1], Ferreira VS[1], Vicentini FTMC[2], Vilela FMP[2], Zarpelon AC[3], Fonseca MJV[2], Baracat MM[1], Georgetti SR[1], Verri Jr WA[3], Casagrande R[1] [1]UEL – Ciências Farmacêuticas, [2]FCFRP-USP – Ciências Farmacêuticas, [3]UEL – Ciências Patológicas

Introduction: UV radiation-induced skin damages may results in immunosupression, aging and photocarcinogenesis. Pyrrolidine dithiocarbamate (PDTC) is a potent antioxidant with strong anti-inflammatory activity and participates in many events that involve the participation of free radicals and cytokines. This study was designed to examine the potential of PDTC to ameliorate the damages caused by UVB exposure in hairless mice. **Methods**: The animals were divided in five groups (n = 5): non-irradiated control, irradiated control and three group of irradiated and treated with PDTC (10, 30 and 100 mg/kg). PDTC was applied intraperitoneal 1 h before the irradiation (4.14 J/cm^2) and 8 h after the first dose. The UVB source used was a Philips TL/12 RS 40W with a peak emission at 313 nm. The animals were terminally anesthetized 2h (cytokines) and 12 h (others tests) after the end of the irradiation. The skin edema was measured as an increase in dorsal skin weight. The UVB-induced leukocyte migration was evaluated using the myeloperoxidase (MPO) spectrophotometric assay. IL-1β levels were determined by an enzyme-linked immunosorbent assay (ELISA). Sodium dodecyl sulphate polyacrylamide gel electrophoresis substrate-embedded enzymography was used to detect enzymes with gelatinase activity. The reduced glutathione (GSH) levels was determined by the 5.5'dithiobis (2 nitrobenzoic acid) (DTNB) spectrophotometric assay. Data were statistically analyzed by one-way ANOVA followed by Bonferroni's t test, and considered significantly different when $p<0.05$. The Ethics Committee on Animal Research of the Universidade Estadual de Londrina approved this study (Registered under the number CEEA 85/10, process n° 33631.2010.82). **Results and Discussion**: Skin edema (52%), MPO activity (77%), IL-1β production (83%) and metalloproteinase 9 (MMP-9, 82%) activity increased as well as GSH levels reduced (55%) after UVB irradiation compared to non-irradiated mice. The treatment with PDTC 10, 30 and 100 mg/kg significantly reduced UVB irradiation induced increase of skin edema by 60%, 48% and 60%; MPO activity by 43%, 41% and 54%; IL-1β production by 43%, 48% and 47%; MMP-9 by 11%, 0%, 25% as well as prevented GSH reduction by 0%, 51% and 84%, respectively. PDTC can act by direct scavenging of ROS by the dithiocarboxy group, and chelating activity for heavy metal ions that may catalyze formation of reactive oxygen species. Nevertheless, the exact anti-inflammatory mechanisms of action of PDTC are not completely understood, but are related to inhibition of nuclear factor kappa B (NF-κB) activation and antioxidant properties. **Financial support**: CAPES, CNPq, Fundação Araucária and UEL.

04.008 Activity of adenosine deaminase (ADA) as a biochemical marker of inflammatory response in patients with visceral leishmaniasis (kala-azar). Cavalcante IJM[1], Galvão LM[1], Nunes MO[1], Gonçalves RP[2], Vale MR[1] [1]UFC – Physiology and Pharmacology, [2]UFC – Clinical and Toxicological Analysis

Introduction: Adenosine is an important endogenous anti-inflammatory agent that has suppressive action on virtually all cells of the immune system. Adenosine deaminase (ADA, EC 3.5.4.4) is the major enzyme that catalyses the deamination of adenosine (or 2'-deoxy-adenosine) producing inosine (or 2'-deoxy-inosine) and ammonia. In humans, ADA is expressed by two isoenzymes (three isoforms): free ADA1 (ubiquitous), ADA1-dipeptidyl-dipeptidase IV (CD26) and ADA2 (present only in monocytes / macrophages). ADA levels are increased in some inflammatory and infectious diseases such as tuberculosis, typhoid fever, viral hepatitis and AIDS. The Kala-azar (Visceral Leishmaniasis) is a parasitic disease that presents itself as a serious public health problem in many parts of the world. In Brazil, visceral Leishmaniasis is endemic, with records of frequent outbreaks, being distributed in 19 states and four of the five regions. This work intends to analyze the levels of ADA and its isoenzymes in plasma of patients with this disease. **Methods**: The samples with serologic testing (K39) positive for Leishmaniasis were collected in a reference hospital for infectious diseases which treats patients from all regions of the State of Ceará. The ADA activity in plasma was determined by the method of Giusti (1974) using adenosine and 2'-deoxi-adenosine as substrate. For the discrimination of ADA isoenzymes agarose gel electrophoresis was used and the assays were performed in presence of 0.1 mM EHNA (specific inhibitor for ADA1). The experimental protocols were submitted and approved by the Ethics in Research Protocol No. 029/2010 (St. Joseph Hospital of Infectious Diseases). **Results:** ADA activity increases significantly in patients with Kala-azar (154.5 ± 17.5 U / L) when compared with controls (19.7 ± 1.1 U / L). The increase of ADA activity in plasma of patients seropositive for Leishmaniasis is mainly due to the ADA2 isoenzyme. **Discussion**: The determination of ADA activity is an important biochemical test that can be used for laboratory evaluation of the inflammatory response in patients with Kala-azar. The isoenzyme ADA2 presents increased levels in plasma of patients with Kala-azar, and this finding is consistent with the fact that the clinical course of infection is mediated by the monocyte / macrophage system. Acknowledgements: We would like to thank CAPES and FUNCAP for **Financial support**.

04.009 Evaluation of antinociceptive and anti-inflammatory activity of new substances derived from isatin. Sardella TB[1], Silva BV[2], Pinto AC[2], Fernandes PD[1]
[1]ICB-UFRJ, [2]UFRJ – Chemistry Institute

Introduction: Isatin (1H-indole-2,3-dione) was first obtained by Erdman and Laurent in 1841 as a product from the oxidation of indigo by nitric and chromic acids. The synthetic versatility of isatin has stemmed from the interest in the biological and pharmacological properties of its derivatives. In nature, isatin is found in plants of the genus Isatis, in the specie *Couroupita guianensis* Aubl., and in humans as it is a metabolic derivative of adrenaline[1]. Some synthetic analogues have been identified as inhibitors MAO[2] and several others have been studied and also showed analgesic, antipyretic and anti-inflammatory effect[3]. The aim of this work was to evaluate the antinociceptive and anti-inflammatory activity of new substances derived from isatin. **Methods:** Isatin (ISA008) and it analogues (ISA017 and 155) were diluted in distilled water and administered orally in male Swiss mice (20 -25g), at doses of 0.1, 1 or 10 mg/kg. The antinociceptive and anti-inflammatory effects were evaluated in formalin test[4] after intraplantar injection of formalin (2.5%, 20 µl). The time (in seconds) that the animals remained licking the injected paw was recorded over the first 5 min (1st phase) and between 15 and 30 minutes (2nd phase). The protocol for the use of animals received the number ICBDFBC015. Statistical analysis was performed by ANOVA and Bonferroni's test (* $p < 0.05$). **Results and Discussion:** Pre-treatment of mice with 0.1, 1 and 10 mg/kg of ISA008, 017 or 155 significantly inhibited the first and second phases of licking response induced by formalin (First phase: control = 52.4±2.6; ISA008: 0.1 mg/kg = 42.6±1.2*; 1.0 mg/kg = 33.0±2.9*; 10 mg/kg = 38.6±6,4*; ISA017: 0.1 mg/kg = 37.9±1.4*; 1.0 mg/kg = 30.1±6.3*; 10 mg/kg = 77.5±4.7*; ISA155: 0.1 mg/kg = 30.9±1.2; 1 mg/kg = 32.2±2.9*; 10 mg/kg = 27.8±4.4*. Second phase: control = 231.9±3.6*; ISA008: 0.1 mg/kg = 187.1±6.8*; 1.0 mg/kg = 141.9±5.9*; 10 mg/kg = 149.5±5.6; ISA017: 0.1 mg/kg = 164.4±3.9*; 1.0 mg/kg = 174.6±15.5*; 10 mg/kg = 77.5±4.7*; ISA155: 0.1 mg/kg = 157.1±3.9*; 1 mg/kg = 146.6±12.7*; 10 mg/kg = 81.4±8.6*). In the first phase is the activation of afferent C fibers, resulting from direct stimulation of nociceptors in formalin. Inhibition of the 2nd phase of the formalin property indicates a potential anti-inflammatory probably resulting ISA's ability to inhibit the formation and/or release of the products of cyclo-oxygenase or lipoxygenase and other pro-inflammatory mediators such as bradykinin, serotonin, and histamine. New analogues of isatin presented anti-inflammatory and anti-nociceptive effects in experimental animal models and suggest that these substances could be new candidates to prototypes of new drugs. **References:** [1]PINTO, A.C. The Chemistry of Isatins: a Review from 1975 to 1999. *J. Braz. Chem. Soc*., 12:273, 2001.; [2]MEDVEDEV, A.B. Inhibitory potency of some isatins analogues on human monoamine oxidase A and B. *Biochem. Pharmacol*, 44:590, 1992.; [3]SRIDHAR, S. Synthesis and pharmacological activities of hydrazones, schiff and mannich bases of isatin derivates. *Biol. Pharm. bull.* 24:1449, 2001.; [4]HUNSKAAR, S. Dissociation between antinociceptive and anti-inflammatory effects of acetylsalicylic and indomethacin in formalin test. *Pain*. 25:125, 1996. **Financial support:** CAPES, CNPq, FAPERJ, and IVB.

04.010 Anti-inflammatory evaluation of the extract from Saracura-mirá. Almeida TS[1], Santos SCM[1], Simen TJM[2], Finotelli P[2], Oliveira DR[2], Leitão SG[2], Fernandes PD[1]
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Introduction: All over Amazon's region, the Saracura-mirá (SM, *Ampelozizyphus amazonicus* Ducke) is extremely important to prevent or cure Malaria. From its barks and roots is prepared an aqueous beverage with abundant foam and bitter taste, similar to beer, which is locally called "cervejinha"1. A recent study shows 48.4% of saponins in an aqueous extract of SM and a bigger abundance of ions K^+, Mg^{2+}, Ca^{2+} and $Fe^{2+/3+}$, compared to other species until now evaluated. The SM is considered a stimulant and energetic plant. Its barks are used to prepare a tonic used to fatigue, in addition to being used as an aphrodisiac. Its roots are considered depurative and are also used in treatment of gastrointestinal disorders, inflammation and fever. Besides these therapeutic properties, SM is also used in treatment of anemia and diabetes. In this work our aim was to evaluate a possible anti-inflammatory activity of the *Ampelozizyphus amazonicus* extract in the model of carrageenan-induced inflammation into the subcutaneous air pouch (SAP). **Methods:** Mice (Swiss, male, 20-25 g, n = 6-8) were orally treated with SM (diluted in distilled water) at doses of 0.3, 1 or 10 mg/kg , one hour before injection of carrageenan (1%) into the SAP. After 24h mice were euthanized and the exsudate from SAP were collected to measurements of cell count, protein, nitric oxide (NO), and cytokines[2]. The protocol for animal use received the number ICBDFBC015. Statistical significance between groups was determined by ANOVA followed by Bonferroni´s test (*$p<0.05$). **Results:** The pre-treatment of mice with the doses of SM significantly reduced cell migration into the SAP. The doses of 0.3, 1, and 10 mg/kg reduced in 3.8%, 48.2%, and 31.7% the cell migration (PBS = 1.3±0.5 x 10^6cel/mL; Carrageenan = 56.8±11.2 x 10^6cel/mL versus 0.3 mg/kg = 54.6±10.5 x 10^6cel/mL; 1 mg/kg = 29.4±4.8* x 10^6cel/mL; 10 mg/kg = 38.8±7.1* x 10^6cel/ml). **Discussion:** The saracura-mirá showed significant anti-inflammatory activity which may explain, at least in part, its popular use. The mechanism by which SM produces its anti-inflammatory effect is still under investigation. **References**: [1]Andrade-Neto VF. Ampelozyziphus amazonicus Ducke (Rhamnaceae), a medicinal plant used to prevent malaria in the Amazon Region, hampers the development of Plasmodium berghei sporozoites**,** *Int J Parasitol*. 38(13):1505, 2008. [2]Martin S. W. The Six-Day-Old Rat Air Pouch Model of Inflammation: Characterization of the Inflammatory Response to Carrageenan, *JPM* 32: 139, 1994. **Financial support:** CNPq, FAPERJ, and IVB.

04.011 CCL3/MIP1alpha induces calcium signaling in cells from rat pre-optic area microcultures but not TNF-alpha or IL-6 synthesis. Soares DM[1], Ott D[2], Souza GEP[3], Roth J[2] [1]USP – Farmacologia, [2]Justus-Liebig University – Veterinary Physiology, [3]FCFRP-USP – Farmacologia

Introduction/Aim: Chemokines are relatively low molecular mass proteins (8–10 KDa) that have chemoattractant effects on many cell types expressing their corresponding receptors, and thus play a critical role in immune surveillance. Although studies have shown that chemokines are pyrogenic when injected into the brain, there is no data indicating which cells or receptors in the CNS chemokines such as MIP-1alpha act to induce fever in rats. Here we investigate which receptor (CCR1, CCR5) of what cell (microglia, astrocytes) of POA the chemokine MIP-1alpha acts through and also if it induces the synthesis/release of pyrogens (TNF-alpha and IL-6) from POA induced by CCL3 / MIP-1alpha. **Methods:** A primary microculture of the POA was established from topographically excised rat pup brain tissue, with cellular identification by marker protein-specific immunocytochemistry. Employing the ratio calcium imaging technique, pyrogen-induced calcium signaling in single POA cells could be characterized. Moreover, the supernatant from the cultures could be analyzed for cytokines measurements. The cultures were stimulated in bolus (100 µl) MIP-1alpha (0.1 or 0.01 µg) or sterile PBS as control. **Results:** In cultures evaluated the total number of cells found was 261 (30.89%) neurons, astrocytes 346 (40.94%) and 238 microglia cells (29.17%). At this moment we have seen that MIP-1alpha, applied in rat pre-óptica area microcultures, is capable of inducing Calcium signaling in astrocytes and neurons. The mobilization of intracellular calcium induced by CCL3 in cell culture was not accompanied by the synthesis release of pyrogenic cytokines IL-6 or TNF-alpha. **Conclusion**: MIP-1alpha induces Calcium signaling on neurons and astrocytes of POA without provoking the synthesis/release of cytokines, what suggests that the fever induced this chemokine can be explained by direct modulating the activity of some cells of POA. **Sponsor**: FAPESP (2008/10323-3). Protocolo no 08.1.1404.53 da Comissão de ética no uso de animais do Campus de Ribeirão Preto da USP

04.012 Chemokines and mitochondrial products activate neutrophils to amplify organ injury during mouse acute liver failure. Marques PE[1], Amaral SS[1], Pires DA[1], Nogueira LL[1], Oliveira AG[1], Soriani FM[2], Teixeira MM[3], Menezes GB[1] [1]UFMG – Morfologia, [2]UFMG – Genética, [3]UFMG – Bioquímica e Imunologia

Introduction: Acetaminophen (APAP) is a safe analgesic and antipyretic drug. However, APAP overdose leads to acute liver failure (ALF), a disorder associated with massive hepatic necrosis and release of intracellular hepatocyte contents (e.g. ATP, , mitochondrial DNA and peptides, pre-formed mediators). In this context, inflammatory mediators (such as chemokines) and spilled intracellular contents are able to induce liver neutrophil recruitment and activation, which could lead to additional liver injury. Thus, our objective was to determine the role of neutrophils in acetaminophen-induced acute liver failure. **Methods:** ALF was induced by APAP overdose (500 mg/kg ; p.o.) in C57BL/6 mice (or $TLR9^{-/-}$). Liver injury was quantified by serum liver enzymes (ALT) and histology. Lung injury was assessed by bronchoalveolar lavage and histology. Neutrophil influx was measured by myeloperoxidase activity and intravital microscopy. Serum mitochondrial DNA (mitDNA) levels were quantified by Real-Time PCR. Liver, lung and serum cytokine levels were measured by ELISA. Also, co-culture of purified human neutrophils and HepG2 cells (hepatocytic lineage) was performed. Drugs and treatments: FPR1 antagonist (BOC-1, 2 mg/kg , i.v.), CXCR2 antagonist (DF2156a, 30 mg/kg , p.o.) or vehicles were given 1 hour before APAP. Neutropenia was induced by anti-Gr1 antibody. Also, serum levels of mitDNA from non-viral fulminant hepatitis patients were quantified. **Results:** During APAP overdose, neutrophils accumulated into the liver and blockage of neutrophil infiltration by anti-GR1 depletion or combined CXCR2/FPR1 antagonism significantly prevented liver injury. No significant reductions were observed using single receptor blockage. Purified neutrophils were cytotoxic to HepG2 cells, and the mechanism of neutrophil killing was dependent on direct contact with HepG2 cells and on CXCR2-FPR1 signaling pathway. Also, in mice and humans, serum levels of both mitDNA and CXCR2-chemokines were increased during acute liver injury. Accordingly, APAP-treated mice presented a marked systemic inflammation, shown by increased levels of serum cytokines and remote lung injury, which was prevented by CXCR2/FPR1 blockage and TLR9 absence ($TLR9^{-/-}$ mice). **Discussion:** We have shown through different pharmacological approaches that neutrophils amplify liver injury during ALF in a CXCR2/FPR1-dependent mechanism. *In vitro* studies confirmed that necrotic hepatocytes stimulated neutrophil killing behavior also via CXCR2/FPR1. In addition, massive liver injury triggered systemic inflammation and remote organ injury, which was prevented by blocking the recognition of necrotic products, mainly mitDNA, chemokines and formyl-peptides. Serum samples from ALF patients confirmed release of mitDNA into circulation following liver injury, which confirms our data and may serve as a diagnostic marker for this disease. In conclusion, pharmacological approaches directed to reduce recognition of necrosis products and liver neutrophil infiltration may consist in a valid alternative to treat acute liver failure. **Ethical Approval:** Human (CEP-FIOCRUZ 22/03); Animal (CETEA UFMG (051/11)). **Financial Agencies:** CAPES, CNPq, FAPEMIG.

04.013 A role for proteinase-activated receptor (PAR)-2 in tryptase-induced eosinophil migration in experimental pleurisy. Matos NA, Matsui TC, Klein A ICB-UFMG – Farmacologia

Introduction: Proteinase-activated receptors (PARs) are G-protein-coupled receptors activated by serine proteinases, through their proteolytic cleavage at a specific site on the N-terminal amino acid sequence of the receptor, and their activation has been shown to be implicated in many hallmarks of inflammation. PAR-2 is a subtype of this receptor, expressed by eosinophils and mast cells and may be activated by trypsin and mast cell tryptase. Allergic diseases have been associated with mast cell degranulation, releasing inflammatory mediators, including proteases as mast cell tryptase. Eosinophils are effector cells of allergic response and have been associated to the development of inflammation. Our goal was investigate the ability of tryptase to induces eosinophil migration in the pleural cavity of mice, and the role of PAR-2 on this recruitment. **Methods:** BALB/c mice (20-25g) were injected via intrapleural (i.pl.) with tryptase (tryp, 30-300 ng/cavity), or with PAR-2 agonist SLIGRL-NH_2 (10-30 µg/cavity) and the number of infiltrating eosinophils was evaluated 24, 48 or 72h after, through the pleural cavity wash. In others experiments, mice were treated with the i.pl. injection of the PAR-2 antagonist ENMD1068 (3µg) 30 minutes before the i.pl. injection of tryp (300ng/cavity). Statistical analyses were performed using One-Way ANOVA followed by Newman-Keuls post-test. Experimental procedures were approved by the local animal ethics committee (CETEA UFMG, protocol number 193/2012). **Results:** tryp or PAR-2 agonist induced a dose-dependent eosinophil recruitment when analysed 24 h after i.pl. injection (PBS, 0.00 ± 0.00; tryp 30 ng/cavity, 1.5 ± 0.6; tryp 100 ng/cavity, 1.2 ± 0.2; tryp 300 ng/cavity, 5.0 ± 0.9* eosinophils x 10^3/ cavity, *P<0.001); (PBS, 0.01±0.01; SLIGRL-NH_2 10 µg/cavity, 0.04±0.04; SLIGRL-NH_2 20 µg/cavity, 0.38±0.07; SLIGRL-$NH_2$30 µg/cavity, 0.71±0.16* eosinophils x 10^3/cavity, *p<0.001). Tryp-induced eosinophil recruitment peaked at 24 and 48 h after (24h: PBS, 0.3 ± 0.2; tryp 300 ng/cavity, 6.0 ± 0.8**; 48h: PBS, 0.3 ± 0.1; tryp 300 ng/cavity, 2.5 ± 0.2*; 72h: PBS, 0.2 ± 0.1; tryp 300 ng/cavity, 1.7 ± 0.5 eosinophils x 10^3/cavity, * P< 0.01, ** P< 0.001) when compared to PBS-treated mice, and ENMD1068 abolished this recruitment (PBS, 1.2 ± 0.04; PBS + tryp 300 ng/cavity, 2.3 ± 0.4*; ENMD 1068 3µg/cavity + tryp 300 ng/cavity, 0.3±0.06* eosinophils x 10^3/cavity, *P<0.001). **Discussion:** PAR-2 activation may be an important step to the eosinophil migration, and tryptase mediates the eosinophil recruitment at least partially through a PAR-2-dependent mechanism. Given that the inhibition of PAR-2 activation may reduce the migration of eosinophils to sites of inflammation, our results suggest that a PAR-2-based therapy may be an useful strategy for the treatment of inflammatory diseases where the infiltrating of eosinophils contributes to the tissue damage. **Financial support:** CNPq/CAPES/ FAPEMIG.

04.014 Lung injury induced by intestinal ischemia reperfusion in obese mice. Fantozzi ET[1], Rodrigues AS[1], Romero DC[1], Breithaupt-Faloppa AC[2], Oliveira-Filho RM[1], Spina D[3], Vasquez YR[3], Tavares-de-Lima W[1] [1]USP – Farmacologia, [2]HC-FMUSP, [3]Kings College London – Pulmonary Pharmacology

Introduction Some studies suggest that obese patients are more likely to have a negative outcome from trauma and sepsis because they already constitutively express many of the inflammatory mediators observed in this condition. However, other studies have reported that mortality is not positively correlated to body mass index (BIM) but instead seems to correlate with morbidity. Interestingly, in some studies a negative correlation between mortality and BMI was found, suggesting a protective role of obesity in cases of acute lung injury. Intestinal ischemia/reperfusion (I/R) causes local and remote injuries, like acute lung injury, that are multifactorial and essentially inflammatory in nature. In this study we have investigated leucocytes migration into the lung induced by I/R in obese mice. **Methods** Female mice (C57Bl6, 21 days old) were fed with a hiperlipidic diet (Pragsoluções Biociências, São Paulo – Brazil) for 90 days. Controls were fed with a standard diet for the same period. The murine model of obesity was validated through the levels of cholesterol and triglycerides in blood, glycemic curve, abdominal fat increase and weight gain. Intestinal ischemia was performed by occlusion of the superior mesenteric artery for 45 min followed by a 2-h reperfusion phase. The systemic inflammation caused by I/R was evaluated by measuring pulmonary myeloperoxidase (MPO) activity and bone marrow leucocytes countings. Cell migration in response to MDC was measured *In vitro* using a modified Boyden chamber. **Results:** Mice fed with the hyperlipidic diet presented a significant weight gain over the period of 90 days in comparison to control lean mice (control = 0.89±0.4; obese = 3.5±0.8 g, n = 10), a slow decay of blood glucose (blood glucose after 60 minutes control = 224.1±14.1; obese = 375.5±30.8 mg/dl, n = 10), higher cholesterol levels (control = 65.5±1.3; obese = 116.9±3.7 mg/dl, n = 7), and increased accumulation of abdominal fat (control = 0.33±0.02; obese = 1.26±0.13 g, n = 10). Triglycerides levels were not altered in groups of animals non-manipulated (control = 42.0±4.0; obese = 60.2±13.0 mg/dl, n = 9), but the animals subjected to the I/R had lower levels of triglycerides (lean I/R = 25.6±3.5; obese I/R = 30.5±4.5 mg/dl, n = 9). The activity of MPO was increased in mice fed with conventional diet and submitted to I/R (control = 1.3±0,1; I/R = 2.6±0.4 450nm, n = 12), and also when fed the hiperlipidic diet (obese = 1.6±0.2; obese I/R = 2.6±0.3 450nm, n = 12). However, the leukocytes countings in the bone marrow were only increased in lean mice subjected to I/R (control = 6.7±0.8; I/R = 14.2±2.5; obese = 6.3±0.9; obese I/R = 4.8±0.8 $x10^6$cells/ml, n = 12). This difference was also observed in the *In vitro* migration assay (control = 9.8±3.0; I/R = 31.5±4.3; obese = 17.5±5.1; obese I/R = 20.3±4.5 $x10^6$cells/ml, n = 8. **Conclusions** Our results suggest that obesity causes an impaired leukocyte migration in vivo and *In vitro*. Ethics Committee approval: nº 040, page 101, book 02. **Financial support**: CNPq and FAPESP - KCL (10/51330-2).

04.015 Anti-inflammatory activities of *Herissantia crispa* L. glycosides isolated. Silva SC[1], Carvalho PRC[1], Oliveira TB[1], Araújo LCC[1], Mota FVB[1], Aguiar JS[1], Silva TG[1], Souza MFV[2], Matias WN[2], Gomes RA[2], Teles YCF[2] [1]CCB-UFPE – Bioensaios para Pesquisa de Fármacos, [2]CCS-UFPB – Ciências Farmacêuticas

Introduction: *Herissantia crispa* L. is a native plant to tropical America, popularly known as "malvarisco". Although there is no indication in folk medicine, the species was chosen according to their chemotaxonomic aspect. It is rich in flavonoids that present various biological activities like immunomodulators, anti-inflammatory, antiviral, antibacterial, hepatoprotective, and antioxidant[1-2]. The objective of this study was to evaluate the anti-inflammatory potential of kaempferol 3-*O*-(6"-*O*-*E*-*p*-coumaroyl)- β-D-glucopyranoside (tiliroside), kaempferol 3,7-di-*O*-α-L-rhamnoside (dhiramnoside) and of the mixture of sitosteryl-3-*O*-β-D-glucopyranoside and stigmasteryl-3-*O*-β-D-glucopyranoside (GM) isolated of the *H. crispa*. **Methods:** To evaluate the anti-inflammatory activity was carried out the experiment in carrageenan-induced air pouch model[3]. Six mice (weight 25-30 g) were used per group. The mice received dhiramnoside, tiliroside and GM glycosides administered orally at a dose of 90 mg/kg , indomethacin (10 mg/kg ; standard) or saline used for the negative control group. One hour after the administration of the compounds, 1 mL of carrageenan solution (1% w / v) was injected into the air pouch. After 6 hours the animals were euthanized in a chamber of CO_2 and the pouch was washed with 2 ml of PBS. A white blood cell count was performed using an ABX Micros 60 hematology analyzer. The exsudate collected was stored in a freezer at -20 ° C for determination of cytokine levels. Quantification of TNF-α and IL-1β these exsudates was determined by sandwich ELISA using mouse specific kits according to the manufacturer's instructions (eBioscience, San Diego, California, USA). The protocol was approved by the Ethics Committee on Animal Experiments of UFPE (23076.050728/2010-84). The results are reported as the mean ± SEM. Multiple comparisons were performed by one-way ANOVA followed by Newman- Keuls test ($p < 0.05$). **Results and Discussion:** dhiramnoside, tiliroside and GM (90 mg / kg) inhibited the migration of polymorphonuclear leukocytes 2.38 ± 0.89 x 10^6 cells 6.70 ± 1.62 x 10^6 cells and 5.24 ± 0 , 49 x 10^6 cells, respectively, compared to the saline control group (11.76 ± 1.30 x 10^6 cells) and indomethacin (3.98 ± 0.73 x 10^6 cells). All compounds tested showed $p < 0.05$ after significant analysis of variance when compared to negative control (saline), whereas when compared to indomethacin, and only dhiramnoside tiliroside showed $p < 0.05$ statistically significant. All compounds tested showed a significant decrease in TNF-α: dhiramnoside (430.7 ± 38.0 pg / mL), tiliroside (502.1 ± 16.4 pg / ml) and GM (486.8 ± 26 8 pg / ml) compared to control (1096.5 ± 0.01 pg / ml). With respect to IL-1β, all compounds decreased production of this cytokine with respect to controls (870.9 ± 0.01 pg / ml): 567.2 ± 97.9 dhiramnoside presented pg / ml, showed tiriloside 694.1 ± 4.07 pg / ml concentrations and GM presented 642.2 ± 36.9 pg / mL. The dhiramnoside was more active in reducing production of both cytokines. The data obtained showed that dhiramnoside, and GM tiliroside isolated from H. crispa showed promising anti-inflammatory properties. The reduction of inflammation is probably due to a modulation of the immune system by decreasing TNF-α and to a lesser extent, decreased IL-1β, however, further studies are needed to clarify the mechanism of action. **Financial support**: Foundation for Science and Technology of the State of Pernambuco (FACEPE). [1]Lima IO, Costa VBM, Matias WN, Costa DA, Silva DA, Agra MF, et al. Biological activity of *Herissantia crispa* (L.) Brizicky. *Rev Bras Farmacogn.*2009 Jan-Mar;19(1B):249-254. [2]Corrêa MFP, Melo GO, Costa SS. Substâncias de origem vegetal potencialmente úteis na terapia da asma. *Rev Bras Farmacogn*. 2008 Dez;18 (Supl.):785-797. [3]Delano DL, Montesinos MC, Desai A, Wilder T, Fernandez P, D'Eustachio P, et al. Genetically Based Resistance to the Anti-inflammatory Effects of Methotrexate in the Air-Pouch Model of Acute Inflammation. *Arthritis & rheumatism*. 2005 Ago; 52(8):2567–2575.

04.016 Hydrogen sulfide modulates reductase glutathione activity and reduced glutathione levels in allergic mice lungs. Mendes JA[1], Campos D[1], Gurgueira SA[2], Vercesi AE[2], Florenzano J[3], Costa SKP[3], Muscará MN[3], Ferreira HHA[1] [1]USF – Alergia e Inflamação, [2]Unicamp – Patologia Clínica, [3]USP – Farmacologia

Introduction: Oxidative stress plays an important role in the pathogenesis of allergic asthma. Hydrogen sulfide (H_2S), endogenously synthesized from the amino acid L-cysteine, may affect oxidative stress as well as airway diseases (Chen and Wang, *Respir Physiol Neurobiol*, 2012 – Epub). Our previous results showed that an H_2S donor, NaHS-treatment, produced a reduction on eosinophil infiltration in the allergic mice lungs at 48h and 144h after OVA-challenged and increased antioxidants enzymes activities, such as, superoxide dismutase (SOD) and peroxidase glutathione (GPx). In addition, these were accompanied by anion superoxide levels and lipid peroxidation decreases in the lungs homogenate. On the other hand, reduced glutathione (GSH) is considered one of the most important antioxidant against free radicals and, therefore, it is a marker for cellular health. The glutathione redox system, composed mainly by GPx and glutathione reductase activity (GR) enzymes, is an important cycle responsible for the maintenance of GSH levels and antioxidant GPx activity (Wang et al. *Exp Physiol* 96:847, 2011). Now, we investigated the effect of exogenous H_2S on the glutathione redox system in the airways allergic inflammation. **Methods:** Female BALB/c mice were subcutaneously sensitized with ovalbumin (OVA) and, 7 days later, were intranasally challenged with OVA twice-daily for 2 consecutive days. Half of the challenged mice were treated with sodium hydrosulfide (NaHS; 14 µmol/kg, i.p.) 30 min before the OVA challenges. At 24h, 48h, 96h, 120h e 144h after OVA-challenge, mice were sacrificed, the lungs were removed and homogenized in buffer phosphate containing a protease inhibitor cocktail to be used for analyze the GR activity by enzymatic kinetic and GSH and oxidized glutathione (GSSG) levels by HPLC. All experiments were approved by the animal ethics committee of USF (license number nº 0021108). **Results:** NaHS-treatment provoked significant increases in GR activity in the airways in approximately 32% and 54% at 48h and 144h after OVA-challenge, respectively, as compared with non-treated allergic (control) mice. An elevation of approximately 128% GSH levels was observed in the lungs of NaHS-treated mice at 144h, compared to control mice. Nevertheless, the GSSG levels were not affected in any time studied. **Discussion:** H_2S donor treatment produced a beneficial effect on the glutathione redox system by increasing the GR enzyme activity as well as the GSH levels in the late phase of airways allergic inflammation in mice. Therefore, this treatment can decrease oxidative stress by increasing this important antioxidant system. Thus, H_2S donors may be a novel therapeutic tool for the treatment of lung diseases characterized by the presence of inflammatory cells and oxidant/antioxidant imbalance. **Financial support:** FAPESP and CNPq.

04.017 Anti-inflammatory evaluation of the extract from flowers of *Couorupita guianensis*. Santos SCM[1], Almeida TS[1], Costa DCM[2], Alviano DS[2], Alviano CS[2], Fernandes PD[1] [1]ICB-UFRJ, [2]UFRJ – Microbiology

Introduction: *Couorupita guianensis* belongs to Lecythidacea family and is also known as "abricó-de-macaco" with distribution in tropical regions of South American. Native amazonian population uses infusions and teas of the leaves, barks and flowers to the treatment of many pathologies as pain, tumors, and inflammations[1]. The aim of this work was to assess the anti-inflammatory activity of the ethanol extract from flowers of *Couorupita guianensis.* **Methods:** The flowers of *C. guianensis* were collected at UFRJ campus, in April/2012. A voucher specimen was deposited at the Herbarium of Biology Institute/UFRJ and received the number RFA 35,645. The ethanol extract was prepared by mixture of flowers (540g) with ethanol (2L). The solvent was evaporated by a rotavapor and the extracted obtained (4.1g) was used to the experiments. Swiss mice (males, 20-25g,n = 4-6) were utilized in models of licking response induced by formalin (2,5%, intraplantar)[2] and in carrageenan (1%)-induced cell migration into the subcutaneous air pouch (SAP)[3]. The animals received oral administration of ethanol extract (10, 30 or 100 mg/kg) 1 hour before the injection of formalin or carrageenan. In the formalin model it was count the time, in seconds, that the animal spent licking the formalin-injected paw. In the SAP model, after 24h of carrageenan injection the mice were euthanized and the exsudate from SAP were collected to several measurements. The protocol for the use of animals received the number ICBDFBC015. Statistical significance between groups was determined by ANOVA followed by Bonferroni´s test (*p<0.05). **Results:** First phase of formalin: control = 48.6 ±7.8 sec; 10 mg/kg = 38.3±12.8 sec; 30 mg/kg = 42.5 ±11.6 sec; 100 mg/kg = 50.4 ±11.2 sec. The second phase: control = 202 ±26sec; 10 mg/kg = 146.8±8.5* sec; 30 mg/kg = 77.9±22* sec; 100 mg/kg = 116.1±38*sec, reducing in 27.3%, 61.4%, and 42.5%, respectively. SAP: PBS = 2,1±0,5x10^6cel/mL; Carrageenan = 79.4±6.9x10^6cel/mL;10 mg/kg = 59.8±22.6x10^6 cel/mL; 30 mg/kg = 63.2±6.3 x 10^6cel/mL; 100 mg/kg = 65.5 ±12.7 x 10^6 cel/mL. **Discussion**: The first phase of formalin is of neurogenic pain and the second is the inflammatory response. The pre-treatment of animals with ethanol extract did not reduce the time that they spent licking the paw in the first phase of formalin model, but it significantly inhibited the second. Despite the effect observed in the second phase of formalin model, none of doses tested significantly reduced the migration of inflammatory cells into the SAP. The mechanism by which it produces the anti-inflammatory effect is still under investigation. **References**: [1]Pinheiro MM. Antinociceptive activity of fractions from Couroupita guianensis**.** Aubl. Leaves**.** *J Ethnopharmacol.* 127(2):407,2009. [2]HUNSKAAR, S. Dissociation between antinociceptive and anti-inflammatory effects of acetylsalicylic and indomethacin in formalin test. *Pain*. 25:125, 1996. [3]Martin S. W. The Six-Day-Old Rat Air Pouch Model of Inflammation: Characterization of the Inflammatory Response to Carrageenan, *JPM* 32: 139, 1994. **Financial support:** CAPES, CNPq, FAPERJ, and IVB.

04.018 Suppression neutrophil migration by NO signaling pathway mediated by anti-inflammatory effect of sulfacted-polyssacaride fraction of extracted from red algae *Hypnea musciformis* in mice. Candeira SJN[1], Sales AB[2], Brito TV[2], Prudêncio RS[2], Vieira Júnior FC[2], Medeiros JVR[2], Souza MHLP[3], Barbosa ALR[2] [1]UFPI, [2]LAFFEX-UFPI, [3]LAFICA-UFC

Introduction: Many algal species contain relatively high concentrations of polysaccharide substances, a number of which have been shown to have anti-inflammatory and/or immunomodulatory activity. The aim of the study was to evaluate the anti-inflammatory effect in mice of a sulfated polysaccharide fraction (PLS) extracted from the algae *Hypnea musciformis* and was to investigate a possible involvement of the NO signaling pathway in this effect. **Methods:** The anti-inflammatory activity of PLS was evaluated using several inflammatory agents (carrageenan, dextran) to induce paw edema and peritonitis in Swiss mice. Samples of the paw tissue and peritoneal fluid were removed to determine myeloperoxidase (MPO) activity, NO_3/NO_2 and IL-1β levels, respectively. Adding to this was also verified through of NO in the modulation of neutrophil migration in carragenaan-induced paw edema or peritonitis. Pretreatment of mice by intraperitoneal administration of PLS (2.5, 5, and 10 mg/kg) significantly and dose-dependently reduced carrageenan-induced paw edema ($P < 0.05$) compared to vehicle-treated mice. Similarly, PLS 10 mg/kg effectively inhibited edema induced by dextran. PLS 10 mg/kg inhibited total and differential peritoneal leukocyte counts following carrageenan-induced peritonitis or paw edema and those effects was reversed by L-Arginine treatment and was recovered by administration of NOS blocker (Aminoguanidine). The project was approved by the Ethics Committee in Research of Universidade Federal do Piauí (Nº. Protoloco: 23111.011979/11-80). **Results and Discussion:** Furthermore, PLS reduced carrageenan-increased MPO activity in paws decreased IL-1β level and increased NO_3/NO_2 level in the peritoneal cavity. Finally, from the results obtained, we conclude that PLS reduces the inflammatory response by modulating neutrophil migration and this seems to be dependent NO pathway. This study aimed to elucidate the anti-inflammatory effect in mice, of the sulfated polysaccharide (PLS) extracted from red seaweed *Hypnea musciformis*. On the paw edema induced by carrageenan, the animals were treated with doses of PLS (2.5, 5 and 10 $mg.kg^{-1}$,i.p.) one hour before carrageenan administration, in this procedure was determined the best dose (10 $mg.kg^{1}$,i.p.) presenting in reduction of edema (95.74% and 84.61% in the 3rd and 4th hours respectively), which was used in subsequent experiments such as edema of dextran, peritonitis, cytokine measurement, myeloperoxidase assays (MPO), Our results suggest that the PLS has anti-inflammatory activity by reducing edema, preventing migration of leukocytes to cavity peritoneal, besides of is involved in the pathway of release of NO in the peritoneum and in paw of mice and act by preventing the release of pro-inflammatory cytokine IL-1β. Those observations indicate that the sulfated-polysaccharide may have clinical potentials for the treatment of inflammation diseases.

04.019 Role of Akt and Erk 1/2 signaling pathways in attenuation of LPS-induced acute lung injury by exogenous leptin, in mice. Landgraf MA[1,2], Silva RC[3], Correia-Costa M[2], Pacheco-Silva A[3], Câmara NOS[2], Landgraf RG[1] [1]Unifesp – Ciências Biológicas, [2]ICB-USP – Imunologia, [3]Unifesp – Medicina Translacional

Introduction: Leptin is an adipocyte-derived hormone that influences a multitude of physiological systems including immunity, inflammation and hematopoiesis. However, role of leptin in pulmonary inflammatory response is still unclear. Lipopolysaccharide (LPS) is an important factor in acute lung injury and airway exposure to LPS in mice induces acute inflammation with recruitment and activation of neutrophils, vascular leakage and bronchopulmonary hyperreactivity. **Objective:** Investigate the role of exogenous leptin in acute lung inflammation induced by LPS. **Methodology:** All procedures were approved by CEP 1666/09 (Federal University of São Paulo). Male C57BL/6 mice at 8-9wk of age were used for each group. Control group was given saline intranasally (i.n.*20mL).* Experimental groups were given leptin (1mg/g/20mL), LPS (1.5mg/g/20mL) or leptin (1mg/g/20mL) and LPS (1.5mg/g/20mL). 24h after instillation the bronchoalveolar lavage was collected to evaluate cellular infiltration in lung. Blood was collected to measure serum insulin. Lungs were harvested for measurement of the mRNA expression of keratinocyte chemoattractant (KC) by real-time PCR and cytokines/chemokines expression by bioplex. Western blot analysis for iNOS protein, Akt and Erk 1/2 phosphorylation was also performed. **Results:** Leptin treatment did not induce any change, when compared to the control group; on the other hand, LPS increased all parameters evaluated. Prior leptin treatment to LPS reduced inflammatory cell infiltration into airways (50%-total cells and 25%-neutrophils), levels of KC mRNA expression (32%), lung tissue cytokine/chemokine expression (80%-rantes; 73%-TNF-a; 70%-IFN-g; 66%-GM-CSF; 72%-VEGF), iNOS protein expression (92%), Akt (45%), Erk1 (68%) and Erk2 (95%) phosphorylation, when compared to the group that received LPS alone. **Conclusion:** These results indicate that exogenous leptin can modulate the LPS-induced acute lung inflammation in mice by down-regulation of proinflammatory cytokines/chemokines and iNOS protein expression. Our results suggest that the downregulation of the Akt and Erk1/2signaling pathways by exogenous leptin is an important mechanism to ameliorate LPS-induced ALI. **Financial support:** FAPESP,CNPq, INCT Complex Fluids and FADA-UNIFESP.

04.020 Cannabinoids inhibit the migration of microglial-like cells in response to the HIV protein Tat through the CB2 cannabinoid receptor. Fraga D[1], Raborn E[2], Ferreira GA[2], Cabral GA[2] [1]UFPR – Farmacologia, [2]VCU – Microbiology Immunology

Introduction: Microglia cells function as the immune surveillance cells in the brain and spinal cord and during insult conditions they develop a reactive phenotype. Upon infection by the human immunodeficiency virus (HIV), microglia secrete a plethora of inflammatory factors, including the virus-specified protein trans-activator of transcription (Tat). This trans-activating protein has been implicated as playing a major role in HIV neuropathology since it elicits chemokines and cytokines from microglia as well as a chemotactic response. It also harbors a beta-chemokine receptor binding motif, articulating a mode by which it acts as a migration stimulus. Since cannabinoids have anti-inflammatory properties, and readily cross the blood-brain barrier, the aim of this study was to investigate the effect of cannabinoids on the migration of microglia toward Tat, a putative early event in HIV neuropathogenesis. **Methods and Results:** Using a mouse BV-2 microglial-like cell model, Tat (25-100 nM), the endocannabinoid agonist 2-arachidonoyl glycerol (2-AG; microM-nM range) and the monoacyl glycerol lipase inhibitor URB602 (microM-nM range) induced a concentration-related increase in the migration of BV-2 cells. In contrast, the exogenous cannabinoid partial agonist delta-9-tetrahydrocannabinol (THC; 1microM – 1nM range) had no effect on migration. Furthermore, THC (1 microM), 2-AG (1 microM) and URB602 (1 microM) exerted a concentration-related reduction in the migration of BV-2 cells that was induced by Tat (50 nM). The CB2 receptor antagonist SR144528 (1 microM), but not the CB1 receptor antagonist SR141716A (1 microM), blocked this reduction of migration produced by THC, 2-AG and URB602 (1 microM). These pharmacological results were confirmed using a CB2R knockdown with small interfering RNA. Knockdown of the CB2R on the microglial cells was checked by western blot analysis. The knockdown of the CB2R reduced the inhibitory effects of THC in the migration of BV-2 cells induced by Tat. Incubation of BV-2 cells with Tat induced a time dependent increase in the expression of chemokine receptors CCR-2, CCR-3 and CCR-5. Concomitant incubation of BV-2 cells with 2-AG plus Tat or THC plus Tat induced a time dependent reduction on the expression of chemokine receptors CCR-2, CCR-3 and CCR-5. In addition to the reduction on the level of the beta-chemokine receptor CCR-3, its intracellular compartmentation was altered to the intracellular level as observed by confocal microscopy. **Conclusion:** Collectively, the results suggest that the CB2R has potential to serve as a molecular target for manipulation of select microglial inflammatory responses elicited by the HIV protein Tat.

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04.021 Role of female sex hormones on cellular recruitment to the lungs after OVA challenge in a murine model of asthma. Golega B, Franco ALS, Oliveira-Filho RM, Tavares de Lima W ICB-USP – Farmacologia

Objective: Clinical and experimental data showed that the variation on circulating levels of female sex hormones (FSH) during the menstrual cycle and their postmenopausal decrease play an important modulating role in asthma. In this study we investigated the effects of FSH on cell recruitment into the lungs in mice that had been ooforectomized after asthma installation. **Methods:** Female Balb/c mice (mean b.w. 20 g) were sensitized with grade V ovalbumin (OVA) and, after 7 days, challenged with OVA for 3 days, once a day for 15 min. One week later, the animals were ovariectomized (OVx) or falsely operated (Sham-OVx). Seven days after OVx, mice were rechallenged with OVA for 3 days, as above. Part of the OVx animals was treated with estrogen or progesterone or both, 4 hours before last three challenges. Twenty-four hours after the last challenge, the animals were killed in excess of ketamine and xylazine and bronchoalveolar (LBA) fluid was performed for further analyses. **Results:** There was an increase in the cellular recruitment to the lungs in mice previously rendered asthmatic and ovariectomized afterwards, and in those treated with estrogen or progesterone. On the other hand, the animals treated with booth, estrogen and progesterone, showed a significant decrease on cellular recruitment into the lungs, reestablishing baseline values. **Discussion:** In our experimental conditions the lack of FSH and the treatment with estrogen or progesterone alone seem to exacerbate inflammatory lung phenomena, mainly by enhancing cellular recruitment into the lungs. Thus, FSH may be importantly involved with the cellular events observed in certain lung pathological (inflammatory) states, e.g. asthma. **Financial support:** FAPESP (2010/51997-7), CNPQ. **Ethic protocol:** Number.118; Page.2; Book.2.

04.022 Suppressive effect of gold nanoparticles on ovalbumin-induced airway inflammation in an asthmatic mouse model. Santos RV[1], Brito FA[1], Ferro JNS[1], Agra LC[1], Santos CE[2], Hickmann JM[2], Giacomelli C[3], Cordeiro RSB[4], Martins MA[4], Barreto E[1] [1]UFAL – Biologia Celular, [2]UFAL – Óptica e Materiais, [3]UFAL – Polímeros e Colóides, [4]IOC – Inflamação

Introduction: Gold nanoparticles (AuNPs) have been proposed for diverse biomedical applications because of their unique surface, electronic, and optical properties. However, nanotechnology has provided a way of producing pure AuNPs, and it shows anti-inflammatory activities and possible pro-healing properties. But, the mechanism of AuNPs on allergic inflammation remains unknown. Aim: This study was aimed to investigate the effects of gold nanoparticles on airway inflammation in a murine model of asthma. **Methods**: AuNPs with an average size of 13 nm in diameter were synthesized following a procedure adapted by Jana et al. (Chem. Mater., v.13, p. 2313, 2001). Male Swiss mice were sensitized with ovalbumin (OVA, 25 µg) (day 0) and boosted (day 14) subcutaneously with OVA (25 µg). At day 21, 22 and 23, mice were challenged with instillation OVA (1% in PBS) and treated 1 h before the OVA challenges by intranasal route AuNPs (400 µg/kg, i.n.) or PBS. On the day of experiments (48 h after the last OVA-challenge), the bronchoalveolar lavage (BAL) was obtained to perform the cell counting. The amounts of cytokines (IL-1β, IL-5, IL-6 and IL-10) in the BAL fluid were quantified by ELISA. In order to identify the changes induced by antigenic provocation, lung segments were processed for H&E staining and histology was evaluated by light optical microscopy. In order to identify the changes induced by antigenic provocation, lung segments were processed for H&E staining and histology was evaluated by light optical microscopy. In other set of experiment, peritoneal macrophages obtained from sensitized animals were used to assess the effect of AuNPs on the production of reactive oxygen species (ROS) stimulated by zymosan *In vitro*. This study was approved by Ethics Committee of Federal University of Alagoas (License no. 9301/2009-96). The results were statistically analyzed using one-way ANOVA followed by Turkey test. The difference were considered significant at $P<0.05$. **Results and Discussion**: Treatment with the AuNPs markedly inhibited airways inflammation noted in sensitized animals subjected to allergen challenge. The increase in the levels of total leukocytes and eosinophils in the BAL fluid were suppressed by the AuNPs. In addition, only the cytokines IL-1β, IL-5, IL-6, IL-10 were inhibited by AuNPs in the BAL fluid. Moreover, the histological analysis showed an attenuation of the eosinophil accumulation in the peribronchial areas after treatment with the AuNPs. We have also found that the increased intracellular ROS levels in peritoneal macrophage after *In vitro* stimulation were decreased by the treatment of AuNPs. These results indicate that gold nanoparticles may attenuate antigen-induced airway inflammation in the murine model of asthma. In addition, In addition, AuNP was able to attenuate the macrophages activation after non-allergic stimulation. These findings may provide a potential molecular mechanism of AuNPs in preventing or treating asthma. **Financial support**: CNPq, CAPES.

04.023 Evaluation of antinociceptive activity and anti-inflammatory of new substances derived from convolutamydine A. Lisbôa YL[1], Gonçalves MR[1], Silva BV[2], Pinto AC[2], Fernandes PD[1] [1]ICB-UFRJ, [2]UFRJ – Chemistry

Introduction: Convolutamydine A(CvA-4,6-dibromo-3hidroxyoxindole) is a substance isolated from the marine bryozoans *Amathia convoluta* and have several biological effects[1].The objective of this work was to evaluate the anti-inflammatory and antinociceptive properties of 3 new analogues synthesized from CvA.
Methods: The anti-inflammatory activity was evaluated by the carrageenan (Carr)-induced inflammation into the subcutaneous air pouch (SAP) and the antinociceptive activity by formalin(intraplantar, 2%)- or capsaicin(intraplantar, 5,2nmol/paw)-induced licking response. Male mice (20-25g,n = 5-8) received oral administration of 158, 160 or 161 (1 or 10 mg/kg) 1h before formalin, capsaicin or Carr. After 24h they were euthanized and the exsudate from SAP were collected to several measurements. Authorization for animals' assays was ICBDFBC015. Statistical analyses was performed by ANOVA/Bonferroni's test (*p<0.05). **Results:** Pre-treatment of mice with analogues significantly inhibited the 2 phases of licking response induced by formalin (control 1stphase = 45±1sec; 158**:** 1 mg/kg = 26.2±2.8*sec; 10 mg/kg = 24.7±3.6*sec;160:1 mg/kg = 20.1±2.3*sec; 10 mg/kg = 22.9±0.9* sec; 161: 1 mg/kg = 28.1±9.8*sec;10 mg/kg = 37±1.2*sec. Control 2ndphase = 141±16.1sec; 158:1 mg/kg = 38.5±4.7*sec;10 mg/kg = 14.9±1.3*sec;160:1 mg/kg = 54.5±2.5*; 10 mg/kg = 14.3±1.2*sec; 161: 1 mg/kg = 108.8±25.4sec; 10 mg/kg = 92.8±3.5*sec) or capsaicin (control = 96.7±5.7sec; 158:1 mg/kg = 49.9±12.9*sec; 10 mg/kg = 63.6±13.3*sec; 160: 1 mg/kg = 60±8.8*sec; 10 mg/kg = 12.7±14.7*sec; 161:1 mg/kg = 43.3±4.73*sec; 10 mg/kg = 61.2±5.8*sec). In the SAP, significantly inhibited the cell migration (control = 63.1.4±12.7x10^{6}cells/mL; 158: 1 mg/kg = 53±9.6x10^{6}cells/mL, 10 mg/kg = 44.1±9.8x10^{6*}cells/mL; 160: 1 mg/kg = 29.4±8.7x10^{6*}cells/mL; 10 mg/kg = 54.7± 8.9x10^{6}cells/mL; 161: 1 mg/kg = 33.6±3.3x10^{6*}cells/mL;10 mg/kg = 48.9±8.8 x10^{6} cells/mL), NO (control = 173±14.68µM; 158: 1 mg/kg = 79.8±7.1*µM; 10 mg/kg = 112.7±42.8µM; 160: 1 mg/kg = 86.6±14.8*µM; 10 mg/kg = 28.8±6.4*µM; 161: 1 mg/kg = 148.6±10.5µM; 10 mg/kg = 41.8±13.1*µM), and TNF-α (control = 639.9±53.3pg/mL; 158: 1 mg/kg = 339.8±21.3*pg/mL; 10 mg/kg = 281±42.2*pg/mL; 160: 1 mg/kg = 391.1± 57.7*pg/mL; 10 mg/kg = 190.7±36.7*pg/mL; 161: 1 mg/kg = 240.3±81.7*pg/mL; 10 mg/kg = 382.1±57.8*pg/mL). **Discussion:** The inhibitory effect in 1st phase of formalin model suggests a direct effect on nociceptive receptors or inhibition the release and/or action of agents such as substance P, glutamate and nitric oxide[2]. The inhibitory effect of the 2nd phase indicates a possible anti-inflammatory activity due to inhibition or formation of metabolites of arachidonic acid as well as others inflammatory mediators[3]. The inhibitory effect against capsaicin-induced licking suggest that the substances tested in this work may be acting on vanilloid receptors. All CvA analogues demonstrated significant anti-inflammatory effect and 160 was the most potent. The mechanism by which CvA analogues develops its effect is still under investigation.
References:[1]Kamano, Y. *Tetrahedron Lett* 36:2783, 1992. [2]Parada, C.A. **Neurosci.** 102:937,2001. [3]Hunskaar, S. **Pain**. 25:125, 1986. [4]Santos, A.R. *Neurosci. Lett* 235:73,1997. [5]Shibata, M. *Pain* 1989. 38:347, 1986. **Financial support:** CAPES, CNPq, FAPERJ, IVB.

04.024 Neutrophil extracellular traps contribute to organ dysfunction during endotoxic shock and sepsis. Czaikoski PG[1], Nascimento DCB[2], Sônego F[1], Castanheira FV[1], Souto FO[2], Sousa RB, Abreu M[3], Alves-Filho JF[1], Cunha FQ[1]
[1]FMRP-USP – Pharmacology, [2]FMRP-USP – Immunology, [3]FMRP-USP – Pathology

Introduction: Neutrophil extracellular traps (NETs) are extracellular structures constituted of a chromatin meshwork decorated with antimicrobial peptides, such as neutrophil elastase and myeloperoxidase. These structures are released via a novel form of cell death called NETosis and contain proteolytic activity that can trap and kill microbes. Recently, it was demonstrated that some protein components in NETs, particularly histones, may lead to host cell cytotoxicity. Indeed, NETs formation inside the vasculature in some diseases has been associated to tissue damage. The aim of this study was to evaluate NETs formation and its contribution to organ dysfunction during experimental endotoxic shock and sepsis and also to verify whether NETs could be used as a novel biomarker of organ dysfunction in septic patients. **Methods and Results:** All experiments were performed according to our institution's ethical guidelines (n° 047/2010). C57BL/6 mice (n = 7) were used to induce endotoxic shock by LPS (15 mg/kg , iv) and sepsis using cecal ligation and puncture (CLP) model. Six and twelve hours after LPS injection or CLP surgery, quantization of serum cell-free (cf)-DNA/NETs, blood urea nitrogen (BUN), glutamic-oxaloacetic transaminase (GOT) and creatine kinase (CK-MB) were performed. To evaluate the role of NETs, mice were treated with recombinant human DNAse (5 mg/kg ,iv) or vehicle at 10 min before, 4 and 8 h after LPS injection. Mice with endotoxic shock and sepsis showed high serum concentration of cf-DNA/NETs when compared with control mice, suggesting intravascular formation of NETs. Moreover, LPS-injected and septic mice showed increased levels of BUN, TGO and CK-MB when compared with control mice. Treatment with recombinant human DNAse decreased serum levels cf-DNA/NETs (6 and 12 hours after treatment). Interestingly, serum BUN, TGO and CK-MB levels (12 hours after treatment) were also decreased in LPS-induced endotoxic shock but not in CLP-induced sepsis after DNAse treatment. Extending to human sepsis, clinical data and plasma samples were obtained from septic patients (n = 33) of Intensive Care Unit (ICU) of Hospital das Clínicas de Ribeirão Preto and healthy controls (n = 5). Plasma levels of cf-DNA/NETs were significantly increased in septic patients compared with healthy controls. The increase of the concentration of cf-DNA/NETs presented a positive correlation with Sequential Organ Failure Assessment (SOFA) score (Spearman r = 0.50, p = 0.002), plasma creatinine (Spearman r = 0.49, p = 0.04) and bilirrubine (Spearman r = 0.46, p = 0.04). **Conclusion:** Our results show that NETs are formed in the vasculature during LPS-induced endotoxic shock, experimental and human sepsis. In addition NETs degradation by DNAse attenuates LPS-induced organ damage. Altogether, our results suggest that NETs are associated with organ dysfunction during endotoxic shock and sepsis. **Financial support:** CNPq, CAPES, FAPESP, FAEPA.

04.025 Inhibitory effect of statin on the microcirculation *in situ*. Ames FQ, Barbosa CP, Bracht L, Estevão-Silva CF, Ritter AMV, Arruda LLM, Cuman RKN, Bersani-Amado CA UEM – Pharmacology and Therapeutics

Introduction: Recent studies performed by our research group have demonstrated that the ezetimibe + simvastatin (Ez+Si) combination was more effective in reducing the inflammatory response in arthritic rats than atorvastatin (At), simvastatin (Si) or ezetimibe (Ez) monotherapy. However, the mechanism responsible for effect remains unknown. This study was focused for investigate the effect of statins on rolling and adherent leukocytes in different periods after the induction of adjuvant arthritis. **Methods:** Arthritis was induced by an intradermal injection of a suspension of *M. tuberculosis* (100 µg) in mineral oil into the plantar surface of the hind paws of Holtzman rats (200-220g). $Si_{40\ mg/kg}$, $Ez_{10\ mg/kg}$, $At_{10\ mg/kg}$ and $Ez_{10\ mg/kg}$ +$Si_{40\ mg/kg}$ were given intragastrically and the treatment began on the day of CFA injection and continued daily up to the 1, 7, 14 and 28th day after arthritis induction. The microcirculation *in situ* was carried out in postcapillary venules of the spermatic fascia, with a diameter of 15-25 micrometers.The number of rolling and adhered leukocytes were determined at 10 minutes-intervals. The protocol was approved by the Ethics Committee on Animal Experimentation of State University of Maringá (062/2008). Data were expressed as mean ± SE, and the significance level was set at $P < 0.05$ (Anova followed by Tukey's test). **Results:** The number of leukocyte rolling and adherent was augmented in arthritic rats, as early as 24 h after the induction of arthritis (rolling / adherent–arthritic rats - 1th day- 333.0±12.2 / 19±0.7; 7th day– 531.0±25.3 / 28.0±1.4, 14th day– 382.0±11.9 / 20.0±0.8; 28th day– 398.0±10.7 / 22.0±0.9), and kept high up to 28th day, compared to non arthritic animals (rolling / adherent– non arthritic rats - 1th day- 228.0±10.0 / 10.0±0.5; 7th day– 232.0±10.2 / 11.0±0.6; 14th day– 235.0±1.3 / 11.0±0.8; 28th day– 244.0±5.1 / 12.0±0.8). The treatment of arthritic animals with statin has significantly reduced the number of rolling and adherent leukocytes in the periods of 7, 14 and 28 days. The effect was similar for all examined drugs (rolling / adherent – treated arthritic rats: Ez -7th day- 294.0+33.2 / 13.0+0.9, 14th day– 194.0+19.4 / 10.0+1.0, 28th day– 280.0+33.0 / 14.0+0.9; Si - 7th day- 301.0+10.8 / 13.0+0.9, 14th day– 183.0+14.1 / 9.0+1.1, 28th day– 268.0+15.9 / 14.0+0.9 ; At - 7th day- 285.0+17.2 / 15.0+1.1, 14th day– 186.0+16.4 / 10.0+1.1, 28th day– 251.0+10.5 / 12.0+0.5; Ez+Si - 7th day- 372.0+23.4 / 17.0+0.9, 14th day– 244.0+23.7 / 12.0+0.5; 28th day– 279.0+17.5 / 14+0.9. Along 24 hours no change was detected in comparison with the control arthritic rats (rolling / adherent – treated arthritic rats - 333.0±12.2 / 19±0.7; Ez- 320.0±30.3 / 17±1.6, Si– 309.0±17.8 / 16.0±0.9; Ez+Si - 304.0±20.9 / 16.0±0.8; At- 319.0±.6.9 / and 17.0±.1.0). **Discussion**: This study pointed out that the treatment of arthritic rats with statins (Simvastatin, Ezetimibe, Atorvastatin and Ezetimibe + Simvastatin) were able to inhibit similarly the rolling and adherent leukocytes, suggesting that this activity may be partially responsible for the anti-inflammatory effect of statins in this experimental model. **Research support**: CNpq/UEM, Fundação Araucária/PR

04.026 The anti-inflammatory compound LASSBIO-930 prevents alveolar bone loss in ligature-induced periodontitis in rats. Silva NLC[1], Maia RC[1], Silva LL[1], Ramos BF[1], Soares MA[1], Cabral MG[2], Abrahão AC[2], Camargo GACG[3], Barreiro EJ[1], Miranda ALP[1], Tributino JLM[4] [1]FF-UFRJ, [2]FO-UFRJ – Patologia e Diagnóstico Oral, [3]PUNF-UFF, [4]ICB-UFRJ

Introduction: Periodontitis (PD) is a chronic inflammatory disease characterized by the periodontal tissue destruction (PTD) and alveolar bone loss (ABL). PD is caused by infiltration of gram negative bacteria in gingival tissue leading to overproduction of proinflammatory cytokines, ciclooxygenase-2 (COX-2) expression and synthesis of prostaglandin E_2 (PGE_2), which activates bone resorption pathways culminating in tooth loss (Noguchi,Period 2000 43:85,2007). Systemic therapy with classic or selective COX-2 nonsteroidal anti-inflammatory drugs (NSAIDs) inhibits experimental PD in rats (Azoubel, Braz J Med Biol Res 40:117,2007). LASSBio-930 is a drug candidate with an anti-inflammatory profile apparently acting via non-selective COX inhibition (Tributino,Bioorg Med Chem 17:1125,2009). Therefore, we investigated the effects of this compound on PTD and ABL in a rat model of ligature-induced PD. **Methods:** Animal protocols were approved by UFRJ ethical animal care committee (DFBCICB044), and 6-8 animals were used per group. PD was induced in male Wistar rats by ligature placement (3-0) around the first lower molars. PD-animals were treated with indomethacin (IND) (14 µmol/kg), LASSBio-930 (100 µmol/kg) or vehicle (**C**) (PBS/1%Tween80/2%DMSO) by oral or intraperitoneal administration, 5 days after the induction of PD and daily until sacrifice (11th day). The sham (**S**) group received only vehicle. The gingival tissues surrounding the molars from the right hemimandibulae were then used to determine myeloperoxidase (MPO) and PGE_2 levels by ELISA. The ABL was determined by the sum of the distances between cement-enamel junctions and the alveolar bone crest of all molar roots. The left hemimandibulae were submitted to histopathological analysis. Results are expressed in mean ± standard error and were statistically analyzed using Student's t test or one-way ANOVA (Sallay,J Period Res 17:263,1982; Crawford,J Period Res 13:316,1978). **Results:** The **C** group showed an enhanced ABL, PGE_2 and MPO levels compared to the **S** group (6.2±0.4 vs. 3.5±0.3 mm; 140.6 ± 7.0 vs. 25.1 ± 29.9 ng/mg of protein (ptn); 1.04 ± 0.18 vs. 0.09 ± 0,08 optical density(OD)/mg of ptn; $p<0.001$ for the three parameters). IND and LASSBio-930 treatment reduced the ABL in 92.4% (3.7±0.1mm; $p<0.001$) and 40.5% (5.1±0.1mm; $p<0.05$) respectively, and PGE_2 levels (4.4±0.7 and 14.5±4.2 ng/mg of ptn, respectively) compared with **C** group. Only IND treatment reduced MPO levels (0.19±0.06 OD/mg of ptn; $p<0.001$). The histopathological analysis showed that the inflammatory infiltrate was reduced in both IND and LASSBio-930 groups. **Discussion:** It is well recognized that both COX-2 up regulation and PGE_2 increased levels have an important role in PD (Offenbacher,J Period 64:432,1993). LASSBio-930 was able to reduce ABL in a well-established model of PD in rats. This effect may be due to the non-selective inhibition of COX, preventing prostanoid formation and reducing the inflammatory infiltrate. In contrast to most NSAIDs that usually cause gastric irritation in long-term treatments, LASSBio-930 did not produce this side effect, as shown previously. Therefore, this compound is a promising candidate to treat PD. **Financial agencies:** CAPES,FAPERJ,INCT-INOFAR.

04.027 Anti-inflammatory activity of new alkaloid isopropyl N-methylanthranilate from the essential oil of *Choisya ternate* Kunth and analogs methyl and propyl N-methylanthranilate. Pinheiro MMG[1], Radulovic NS[2], Miltojevic AB[2], McDermott M[3], Waldren S[3], Parnell JA[3], Boyan F[3], Fernandes PD[1] [1]ICB-UFRJ, [2]University of Nis – Chemistry, [3]Trinity College Dublin – Pharmacy and Pharmaceutical Sciences

Introduction: The infusion of leaves of *Choisya ternate* Kunth (Rutaceae) is popularly employed for their antispasmodic property. A GC-MS analyses of the essential oil of *C. ternate* revealed the presence of isopropyl *N*-methylanthranilate (ISOAN) and other volatiles compounds. **Objectives:** ISOAN and two synthetic analogs, methyl-(MAN) and propyl-*N*-methylanthranilate (PAN), were evaluated for their anti-inflammatory activity in the subcutaneous air pouch (SAP) model in mice. **Methods:** The oil of *C. ternate* leaves, collected at Dublin, was obtained by hydrodestillation. Male Swiss mice (20–25g; n = 6-8) received oral administration of ISOAN, PAN or MAN (1-10 mg/kg) 1h before carrageenan (1%) injection into the SAP. After 24h the mice were euthanized and the exsudate from SAP were collected to measurements of cell count, protein, nitric oxide (NO) and cytokines. The protocol for the use of animals was approved by the Ethical Committee and received the number ICBDFBC015. Statistical significance between groups was determined by ANOVA followed by Bonferroni´s test (*$p<0.05$). **Results:** Anthranilates showed significant anti-inflammatory activity in the doses evaluated, reducing cell migration (control = 18±9x10^6cell/mL; ISOAN: 1 mg/kg = 15±6x10^6cell/mL; 3 mg/kg = 9.7±2*x10^6cell/mL; 10 mg/kg = 4.3±1*x10^6cells/mL; MAN: 1 mg/kg = 17.1±7x10^6cell/mL; 3 mg/kg = 10.7±6x10^6cell/mL; 10 mg/kg = 6.24±0.85*x10^6cells/mL, and PAN: 1 mg/kg = 8.9±5*x10^6cell/mL; 3 mg/kg = 5.3±4*x10^6cell/mL;10 mg/mL = 4.8±0.8*x10^6cells/mL), protein extravased (control = 228±102mg/mL; ISOAN: 1 mg/kg = 286±44; 3 mg/kg = 70±28*; 10 mg/kg = 61±19*mg/mL; MAN: 1 mg/kg = 247.4±70; 3 mg/kg = 106±34*; 10 mg/kg = 64±20* mg/mL; and PAN: 1 mg/kg = 232±85; 3 mg/kg = 6±24*; 10 mg/kg = 57±7*mg/mL), NO production (control = 76±47mM; ISOAN: 1 mg/kg = 91±23mM; 3 mg/kg = 47±21*mM; 10 mg/kg = 15±4*mM; MAN: 1 mg/kg = 108±30mM; 3 mg/kg = 35±11*mM; 10 mg/kg = 23±8*mM; PAN: 1 mg/kg = 93±21mM; 3 mg/kg = 17±5*mM; 10 mg/kg = 27±11*mM), TNF-α (control = 205±58 pg/mL; ISOAN: 1 mg/kg = 155±37 pg/mL; 3 mg/kg = 163±15 pg/mL; 10 mg/kg = 57±34*pg/mL; MAN: 1 mg/kg = 205±59 pg/mL; 3 mg/kg = 130±42 pg/mL; 10 mg/kg = 73±20*pg/mL; PAN: 1 mg/kg = 228±38 pg/mL; 3 mg/kg = 89±6.1* pg/mL; 10 mg/kg = 59±8*pg/mL), and IL1β (control = 878±87 pg/mL; ISOAN: 1 mg/kg = 881±94 pg/mL; 3 mg/kg = 603±61* pg/mL; 10 mg/kg = 279±71*pg/mL; MAN: 1 mg/kg = 640.2±63 pg/mL; 3 mg/kg = 618±41 pg/mL ; 10 mg/kg = 388±38*pg/mL; PAN: 1 mg/kg = 518.6±69* pg/mL; 3 mg/kg = 236.5±49* pg/mL; 10 mg/kg = 271.9±12*pg/mL). **Conclusion:** The results demonstrate that the alkaloids derivatives present a significant anti-inflammatory activity, reducing several parameters of inflammation. The mechanism(s) by which the substances produce its effect are under investigation. Furthermore, the anti-inflammatory action demonstrated in the present study supports, at least partly, the ethnomedicinal uses of this plant. **Financial support:** CAPES, CNPq, FAPERJ, and IVB.

04.028 Nitric oxide and peroxynitrite as signaling agents for NOS-2 expression in vascular smooth muscle cells. Scheschowitsch K[1], Sordi R[1], Moraes JA[2], Barja-Fidalgo TC[2], Assreuy J[1] [1]UFSC – Pharmacology, [2]UERJ – Pharmacology

Aim: Nitric oxide (NO) plays a key role on vascular tonus maintenance. NO can be synthesized by three NO synthases that can be constitutively expressed (c-NOS: NOS-1 and NOS-3) or have its expression induced (NOS-2) by pro-inflammatory agents. During sepsis, a systemic inflammatory disease, a persistent hypotension occurs in part due to the presence of a large amount of NO on vascular bed. Previous results our laboratory show that hypotension and mortality during sepsis is prevented by the early administration of NOS-1 inhibitors, suggesting that c-NOS play an important role in sepsis. Thus, the importance of NO derived from c-NOS and other reactive species in vascular smooth muscle cell activation was investigated *In vitro*, using the cell line of rat aorta smooth muscle cells, A7r5. **Methods:** NO and ROS production were evaluated with the fluorescent probes DAF-FM DA and H_2DCF DA. Cells were stimulated with LPS 1 µg/mL and IFN 200 U/ml (LPS/IFN). In another set of experiments cells were treated 30 min with a NO scavenger (carboxy-PTIO 100 µM) and a NOS inhibitor (7-NI 200 µM) before stimulation. Fluorescence intensity was expressed as fluorescence intensity to control group. NOS content was evaluated by Western blot. Nitrite was assayed by Griess reaction on cell supernatant 48 h after cell stimulation. Immunofluorescence was used to evaluate protein nitration (30 min and 2 h) and NF-κB nuclear translocation. Images were acquired on confocal microscopy (63x immersion, Leica®), quantified by LAS® software and results expressed as relative fluorescence intensity to control group of nuclear content. All results are representative of 3 independent experiments, and statistical comparisons performed by two-way ANOVA followed by Bonferroni test. **Results:** LPS/IFN stimulated A7r5 cells presented an increase in intracellular NO (3.5±0.3 relative fluorescence) and peroxynitrite/hydrogen peroxide (3.4±0.1 RF) content, compared to control (non-stimulated cells) group (0.1±0.0 and 1.9±0.1 RF, respectively). A7r5 control cells express c-NOS, but not NOS-2, which was detected from 12 h after cell stimulation as shown by Western blot. Since LPS induces intracellular ROS production and we have shown that it induces NO as well, we studied the peroxynitrite generation. This hypothesis was confirmed by the reduction in fluorescence intensity of H_2DCF DA probe in the presence of 7-NI (3.4±0.2 versus 2.1±0.1 RF, for LPS/IFN and 7-NI+LPS/IFN group, respectively) and decreased nitrotyrosine immune reaction caused by PTIO and 7-NI. A decrease in NOS-2 expression and nitrite production was also observed in PTIO and 7-NI treated cells. Furthermore, nuclear translocation of NF-kB evaluated 30 minutes after cell stimulation was reduced by PTIO and 7-NI (1.3±1.0 and 1.6±0.1 RF, respectively) compared to stimulated cells (2.0±0.2 RF). **Conclusion:** An early NO pulse derived from c-NOS activity and the subsequent peroxynitrite generation occurs after smooth muscle cell stimulation with LPS/IFN. Together, these species modulate NOS-2 expression through modulating NF-κB nuclear translocation. These results shown, for the first time, the importance of low levels of NO and peroxynitrite as signaling agents in vascular smooth muscle cell NOS-2 expression. **Financial support:** CAPES, CNPQ and FAPESC.

04.029 Regulatory activity of annexin-1 on the model of asthma induced by house dust mite in mice. Trentin PG[1], Souza DM[1], Flower RJ[2], Perretti M[2], Martins MA[1], Silva PMR[1] [1]Fiocruz – Inflammation, [2]The William Harvey Institute – Biochemical Pharmacology

Introduction: Asthma is a chronic inflammatory disease characterized by bronchoconstriction and airways hyperreactivity as well as eosinophil accumulation in lung tissue, mucus secretion and remodeling. During the establishment of an inflammatory process, endogenous mediators are released in order limit the progression of the pathological process and guarantee the homeostasis. Glucocorticoid hormones are recognized as critical on their potent anti-inflammatory activity, which is at least partially dependent on the release of intermediate factors such as the protein annexin-1. Thus, in this study we investigated the regulatory role of annexin-1 (AnxA1) on experimental model of asthma induced by house dust mite (HDM) in mice. **Methods:** AnxA1 null and wild type littermate (Balb/c) mice were sensitized with intranasally instillation of house dust mite (HDM - 25 µg/25 µL), every other day, during 3 weeks. Twenty four hours after the last provocation the analyses were performed and included: i) lung function (resistance and elastance) and airways hyperreactivity to methacholine (3-27 mg/ml) by invasive plethysmography (Buxco System), ii) morphological and morphometric evaluation of peribronchiolar eosinophil infiltration (Sirius red 2.0), extracellular matrix deposition (Gomori trichrome) and mucus production (PAS), and iii) cytokine/chemokine generation (ELISA). All the experimental procedures were approved by the Ethics Committee of Animal Use of FIOCRUZ (License 034/09). **Results:** We noted that stimulation with HDM led to a marked increase in the basal levels of lung resistance and elastance, and that aerozolization with methacholine exacerbated such responses, indicating a clear state of airways hyperreactivity. In parallel, HDM induced lung inflammation, which was characterized by a dramatic increase in inflammatory cell recruitment, including infiltration in the interstitial space, and a marked eosinophil accumulation in the peribronchiolar area. Excessive deposition of extracellular matrix was also noted around the airways. An increased generation of pro-inflammatory and pro-fibrotic cytokines (IL-4, IL-5 and TGFβ) and chemokines (MCP-1, eotaxin-1 and -2) in the lung tissue and of mucus deposition in the airways was also detected in the HDM-challenged mice. Mice deficient in AnxA1 displayed higher levels of airways hyperreactivity to methacholine and increased leukocyte infiltration including peribronchiolar eosinophils accumulation. In a parallel analysis, higher levels of lung tissue cytokine and chemokine generation as well as airways remodeling and mucus production were detected in the AnxA1 null mice. **Conclusion:** Together our findings show that AnxA1 null mice were more responsive to antigenic stimulation with HDM as compared to wild type mice, which indicates that AnxA1 appears to exert an important regulatory role on the response murine allergic inflammation. This may be an indicative that AnxA1 may contribute to development of new approach for treatment of allergic diseases as asthma. **Financial support:** FIOCRUZ, CNPq, CAPES, FAPERJ (Brazil).

04.030 The N-acylhydrazone derivative LASSBIO-897 suppresses lung inflammation caused by silica particles in mice. Arantes ACS[1], Ferreira TPT[1], Ciambarella BT[1], Trentin PG[1], Ramos TJ[1], Amigo YS[1], Barreiro EJ[2], Fraga CAM[2], Martins MA[1], Silva PMR[1] [1]IOC – Inflamação, [2]UFRJ – Substâncias Bioativas

Introduction: Silicosis is an occupational disease caused by prolonged inhalation of dust containing free crystalline silica particles, which is characterized by an intense pulmonary inflammation with formation of fibrotic nodules. Estimates indicate that silicosis kills million of workers every year worldwide and, in Brazil more that 6 million of workers are at a risk of silicosis. As there is no effective treatment for fibrotic diseases, in this study we investigated the effect of the 1,3-N-acylidrazonic benzodioxolic derivative LASSBio-897on the experimental silicosis in mice. The effect of the compound was also evaluated in some target cells *In vitro*. **Methods:** Swiss-Webster mice (CEUA- License 034/09) were anesthetized and intranasally instilled with silica (10 mg/50 uL) and analyzes performed after 28 days. The animals received daily administration of LASSBio-897 (1.25 – 5 mg/kg, po) for 7 days, starting 21 days after stimulation with silica. The evaluation of pulmonary mechanics was performed by invasive whole body plethysmography (Fine point - Buxco System), in the absence or the presence of methacholine (3-27 mg/mL). Classical histological techniques were used and included morphology and morphometry (H&E and picrus Sirius) and immunohistochemistry. Quantification of collagen deposition and of cytokine/chemokine generation was made by ELISA. Silica-activation of lung fibroblasts, macrophages and epithelial cells was also evaluated *In vitro*. **Results:** Therapeutic treatment of silicotic mice with LASSBio-897 inhibited the reduction in the lung function (resistance and elastance) and airways hyperreactivity to methacholine, as well as collagen deposition, granuloma formation and cytokine/chemokine generation in the lung tissue. LASSBio-897 also suppressed the increased expression of α-SMA and F4/80 in the silicotic mice, indicating that fibroblasts and macrophages seem to be important targets for the compound, respectively. The evaluation of LASSBio-897 effect on the functionality of target cells *In vitro* showed that, at the concentrations varying from 0.01 to 10 μM, the compound inhibited silica-activated alveolar macrophages (TNF and nitric oxide generation), and IL-13-induced lung fibroblast proliferation and collagen production. No effect was noted in the case of silica-induced epitelial cell IL-8 production. **Conclusion:** Our results show that compound LASSBio-897 inhibited the inflammatory response and granuloma formation caused by silica particles in the lungs of mice, by a mechanism dependent on its suppressive effect on macrophages and fibroblasts but not on epithelial cells. **Financial Support**: FIOCRUZ, INCT-INOFAR, PRONEX 2009, CNPq and FAPERJ.

04.031 Clinics, **gastroscopical and histopathological findings after 28 consecutive days of meloxicam and carprofen treatment**. Portugal MNM[1], Erthal E[1], Alcântara CF[1], Knopf T[1], Benevenho AC[2], Miara LC[1], Quitzan J[1], Pimpão CT[1] [1]PUCPR – Agricultural Sciences and Veterinary Medicine, [2]USP – Agricultural Sciences and Veterinary Medicine

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in veterinary medicine in the treatment of chronic and acute pain; however its indiscriminate use has been linked to *Gastric Erosive-ulcerative Disease.* This study was approved by the Research Ethics Committee of PUCPR, protocol 568/2010. Therefore, the aim of this study was to evaluate the adverse effects of NSAIDs (carprofen and meloxicam) in dogs. Sixteen dogs were randomly divided into three groups: group 1 (negative control - no medication, n = 4), group 2 (4.4 mg carprofen / kg, PO, n = 6) and group 3 (meloxicam 0.1 mg / kg, PO, n = 6). All groups underwent the same routine of treatment, food, clinical, endoscopic and laboratory for 28 days. Data were analyzed by Student t test, and Kruskal-Wallis test, followed by Dunn test. In relation to laboratory exams of liver function, urinalysis and complete blood count, it is possible to affirm that there were no changes among groups, as well as the comparison values within groups before and after treatment, remained in accordance to the normal limits. Dogs treated with carprofen had isolated episodes of vomiting and diarrhea during the treatment, whereas the dogs treated with meloxicam presented diarrhea, melena, hematochezia, starting by 14th day of treatment. Dogs of the three groups evaluated showed macroscopic and microscopic gastric mucosal variations, from mild to moderate level, without establish significant difference ($p > 0.05$) between groups. It was possible to maintain the continuous administration of carprofen for 28 consecutive days, while group meloxicam showed clinical signs of gastropathy after the 10th day of treatment.

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04.032 Anti-inflammatory and antinociceptive effects of ATB-346, a gastric sparing hydrogen sulfide-releasing naproxen, in rats with carrageenan-induced knee joint synovitis. Ekundi-Valentim E[1,2], Rodrigues L[1], Santos KT[1], Teixeira SA[1], Wallace JL[3], Costa SK[1], Muscará MN[1] [1]USP-ICB – Farmacologia, [2]ISCISA-UAN, [3]Farncombe Institute-McMaster University

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly prescribed agents in arthritic patients, although the gastric effects limit their long-term use. Considering the gastric safeness of H2S-releasing NSAIDs (FASEB J 2006; 20: 2118), in addition to the beneficial effects of H2S in rat synovitis (Br J Pharmacol 2010; 159: 1463), we evaluated NAP and ATB-346 in rats with Carrageenan (CGN) - induced sinovitis. Male Wistar rats (180 - 200 g) were obtained from our animal house care facilities and used in this study. All the experimental procedures were in accordance with the ethical principles for animal research established by the local ethics committee (protocol nº 64, page n º 46/2007). Anesthetized (halothane + O2) rats were pre-treated with either NAP (0.3, 1, 3 or 10 mg/kg) or equimolar ATB-346 doses (0.48, 1.6, 4.8, or 16 mg/kg) 30 min before the i.art. injection of 7.5 mg of CGN. Joint swelling and pain score were assessed 1, 3 and 5 h after CGN, and tactile allodynia (von Frey filaments) after 2 and 4 h. At the end, joint cavity lavages were collected for leukocyte counting. The drugs (at the highest doses) were also tested for their gastric effects by evaluating mucosa integrity (macroscopical) and neutrophil recruitment (as myeloperoxidase – MPO activity). CGN induced edema, pain, tactile allodynia and leukocyte infiltration into the joint cavity. Edema and pain score (at 3 and 5 h after CGN) were reduced by both NAP and ATB-346 at the two highest doses ($P<0.001$). Tactile allodynia (4 h after CGN) was also similarly inhibited (by ~45%; $P<0.001$) by both NAP (at 1, 3 and 10 mg/kg) and ATB346 (at 1.6 and 4.8 mg/kg), as well as leukocyte infiltration. No macroscopical lesions of the gastric mucosa were observed in any of the groups; however, NAP (but not ABT-346) increased gastric MPO (~130%). We conclude that the H2S-releasing moiety in the ATB-346 structure protects the gastric mucosa from the parent drug-induced side-effects and does not reduce NAP efficacy for the control of local inflammation and hyperalgesia in rats with CGN-induced synovitis. **Financial support**: FAPESP, CNPq, CAPES, Agostino Neto and McMaster University.

04.033 ***Achyrocline satureoides*** **(LAM) D.C. extract treatment prevents neutrophil migration in air pouch model**. Barioni ED[1], Santin JR[1], Shimada AL[1], Rodrigues SF[1], Machado ID[1], Ferraz-de-Paula V[1], Niero R[2], Andrade SF[2], Farsky SHP[1] [1]USP – Clinical and Toxicological Analyses, [2]Univali

Achyrocline satureoides (Lam) D.C, popularly known as "Marcela", is widely used to treat several illnesses. The aim of this study was to elucidate the anti-inflammatory mechanisms of the *A. satureoides* inflorescences, mainly on neutrophil function.
Male Wistar rats were orally treated with 100 mg/kg of the crude hydroalcoholic extract of *A. satureoides*, and 1h after, the inflammatory process was induced by LPS (0.5 mg/mL PBS; 2mL) injection into the dorsal subcutaneous tissue (air pouch). Blood and inflamamtory exudate was collected 1h later. Control animals received vehicle. The total and differential cell number (Neubauer and Panoptic dyed smears) and the concentration of the chemotactic mediators (ELISA) in the exudate were quantified. Membrane receptors were measured in peripheral neutrophils by flow cytometry. In other set of animals, the LPS-induced (topical application; 30 µg/40µL PBS) leukocyte-endothelial cell interaction in the mesenteric microcirculation was evaluated by intravital microscopy 2h after the treatments. All experiments were approved by the Ethics Committee on Animal Use and Care (Protocol CEUA/FCF/334). The results showed that oral administration of *A. satureoides* reduced both neutrophil migration into the air pouch and LTB4 and CINC-1 concentrations in the exudate; reduced the expression of TLR4 and reversed the increased expression of CD62L and the reduced expression of CD18 in circulating neutrophils and reduced the number of adhered leukocytes on post-capillary venules. Taken together, our data show that *A. satureoides* oral treatment prevents the LPS-induced neutrophil migration to the inflammatory site, by affecting the adhesion molecule expression in neutrophils and by reducing the secretion of chemotactic mediators. **Financial support**: FAPESP (2011/15115-2); CAPES

04.034 Polychlorinated biphenyl 126 inhalation alters metabolic parameters and inflammatory markers in rats. Shimada ALB, Cruz WS, Nakasato A, Farsky SHP
USP – Clinical and Toxicological Analyses

Polychlorinated biphenyls (PCBs) are persistent organic pollutants and ubiquitous environmental contaminants that resist to degradation and accumulate in the food-chain. PCB126 was widely used in industrial process and is the most potent aryl hydrocarbon receptor agonist and the most toxic PCB in this group. Once respiratory tract is an important point of entry of environmental pollutant exposure, this work aimed to investigate the systemic effects caused by PCB126 inhalation in rats. Male Wistar rats were exposed to PCB126 0.1; 1 or 10μg/kg for 15 days by nasal instillation. Control animals were exposed to vehicle (saline + 0.5% DMSO). Five hours following the last exposure, animals were killed and the number of total and differential circulating and total bone marrow cells were evaluated. The expression of adhesion molecules on circulating leukocytes membranes was evaluated by flow cytometer. In serum samples, the biochemical, lipid and cytokine profiles were measured. Another group of exposed animals was submitted to an intravenous glucose tolerance test. All the experiments were conducted according to Ethics Committee in Animal Experiments approved by protocol number CEUA/FCF/315. While no alterations were observed after 0.1 or 1μg/kg PCB126 exposure in rats for 15 days, changes in the following parameters were observed after 10μg/kg PCB126 exposure for 15 days: 1) Body weight increase along with no change in the food intake, but paralleled by an increase in the liver weight; 2) Reduced number of total circulating leukocytes and bone marrow cells. Reduction in total leukocyte count was due to a low lymphocyte count; 3) Reduced expression of CD62L on circulating neutrophils and lymphocytes at basal conditions; 4) *In vitro* fMLP stimulation impaired CD62L, CD18 and CD31 expression in these cells; 5) Alteration of the lipid profile. No changes were observed in serum TNF-α, IL-1β, IL-6; glucose and aminotransferases levels, or on the glucose tolerance. Taken together, our data indicates that PCB126 inhalation alters some metabolic parameters, and the cell adhesion molecules expression on peripheral leukocytes. Thus, both the body homeostasis and the immune response can be affected by PCB126. **Financial support**: FAPESP (Processes no. 2011/09677-8 and no. 2012/02994-0).

04.035 Exposure of extracellular *Mycobacterium tuberculosis* to isoniazid decreases macrophage activation during infection. Yamashiro LH[1], Souza NM[2], Eto C[2], Báfica A[1] LiDI-UFSC – Farmacologia

Introduction: Tuberculosis (TB) is one of the most devastating infectious diseases worldwide and it is caused by intracellular pathogen *Mycobacterium tuberculosis* (Mtb). However, during active TB, both intra and extracellular bacteria are found in the lesions, raising the question on what are the mechanisms by which Mtb growth is controlled by antibiotics when macrophages uptake drug- exposed extracellular pathogen. Therefore, we hypothesized that exposure of extracellular Mtb to antibiotics influences bacteria-macrophage interactions, such as infectivity and cellular activation. To test this hypothesis, we have utilized an *In vitro* system in which the bacterium has been exposed to low concentrations of isoniazid (INH), a major anti-TB drug, and macrophage infectivity experiments performed. **Methods:** The virulent Mtb laboratory strain H37Rv was exposed to low concentrations of isoniazid (0.01, 0.1 or 1.0 μM) for 24h. Following bacteria washes, bacterial growth was determined after plating cellular extracts onto 7H10 medium and counting of colony forming units (CFU). TNF and Nitrite concentrations were determined in murine bone marrow- derived macrophage supernatants by ELISA and Griess reaction, respectively (CEUA approved protocol PP 00517). **Results:** At early time point (4h) after infection, no difference in CFU counting was observed between the untreated and treated group. Similar numbers of bacteria were also measured at 24h p.i. in all groups, except in Mtb treated at 1.0 μM INH, which displayed a 1.6 fold decreased survival when compared to untreated groups. Surprisingly, despite to the comparable levels of CFU observed in the treated and untreated groups, mycobacterial exposure to minimal concentrations of INH rendered macrophages to undergo a more decreased classical activation phenotype as measured by TNF as well as nitrite levels in cell supernatants. These results suggest that pre exposure of Mtb to INH decreases macrophage activation enhancing bacteria survival. **Conclusions and perspectives:** We speculate that INH influences Mtb cell wall structure modulating macrophage responses towards infection, reducing its capacity to kill the pathogen. Experiments to address the mechanisms by which INH pre treatment blocks Mtb-induced macrophage activation are in progress. **References:** Hoff, D.R. et al., Plos One, 2011. Cade, C.E. et al., Protein Science, 2010. **Financial support:** CAPES, CNPq, NIH, HHMI.

04.036 Effect of pravastatin on aggregation and in the number of circulating platelet in non-treated and lipopolysaccharide-treated rats. Naime ACA, Lopes-Pires ME, Mendes CB, Landucci ECT, Antunes E, Marcondes S Unicamp – Farmacologia

Introduction: The most severe septic responses can be reproduced by lipopolysaccharide (LPS) injection such as decrease of circulating leukocytes and platelets, along with increased production of inflammatory mediators. Platelets are believed to take part in the phatophysiology of sepsis, but data are still conflicting. In the last years, reports have demonstrated the antiinflammatory and antiplatelet effects of statins. Therefore, we decided investigate the effect of pravastatin, a hydrophilic statin, on platelets of non-treated and lipopolysaccharide-treated rats. **Methods:** The present study was approved by the Committee for Ethics in Animal Research (State University of Campinas – UNICAMP, protocol number 2516-1). Male Wistar rats (250-320 g) were separated in two different groups. In the first group, rats were treated with saline (once a day, by oral gavage, for 7 days) and in the second group, rats were treated with pravastain 20 mg/kg (once a day, by oral gavage, for 7 days). In the sixth day, rats of both groups received a single injection of saline or LPS (from *E. coli*, 1 mg/kg) and after 48h arterial blood was collected in ACD-C (9:1 v/v). Platelet and total leukocyte counts were carried in Newbauer chamber. Platelet aggregation was measured in a two channel aggregometer and the assays were carried using ADP (5 μM). **Results:** The counts of total leukocytes in arterial blood of rats treated with saline and injected with LPS were significantly higher compared with control animals, while the platelet number was significantly reduced. Treatment of rats with pravastatin significantly reduced the counts of both platelets and leukocytes compared to the control (reduction of 32% and 56% in leukocytes and platelets counts, respectively). In rats injected with LPS, pravastatin reduced the leukocyte number to the control values and increased the number of platelets (control = $9x10^8$ platelets/ml, LPS-injected rats = $3x10^8$ plateles/ml and LPS-injected rats and treated with pravastatin = $6.3x10^8$ platelets/ml). *In vitro* addition of ADP produced significant washed platelet aggregation in saline-treated rats (52±5% of aggregation, n = 6). Platelet aggregation was significantly reduced by pravastatin treatment (38% of reduction) and by LPS injection (67% of reduction) compared to the control group. However, when the rats were pre-treated with pravastatin and injected with LPS the platelet aggregation was very similar to the saline-treated group (52±5% and 42±8% of aggregation, respectively). **Conclusion:** Pravastatin prevented the inhibitory effect of LPS on platelet aggregation. In addition, this statin brought the circulating leukocytes to the normal counts and significantly increased the platelet number in LPS-treated rats. These results showed that pravastatin restores the platelet response and improves the inflammatory condition in the experimental sepsis. **Supported by:** CNPq

04.037 Regulation of purinergic signaling in endothelial cells during chronic inflammation. Oliveira SDS[1,2] , Oliveira NF[2], Meyer-Fernandes JR[3], Coutinho-Silva R[1], Silva CLM[2] [1]IBCCF-UFRJ, [2]ICB-UFRJ – Farmacologia Bioquímica Molecular, [3]IBqM-UFRJ

Introduction: Schistosomiasis is an intravascular disease related with vascular alterations and chronic inflammation. The aim of this study was to evaluate the purinergic signaling in mesenteric endothelial cells (MECs) from *S. mansoni*-infected mice with special interest in the P2X7 receptor (P2X7R) function and expression, ectonucleotidases activity and leukocyte adhesion mediated by P2Y1 receptors (P2Y1R). **Methods:** *S. mansoni*-infected and control mice were used (ethics committee DFBC-ICB-011) to obtain primary cultures of MECs as previously described (Silva *et al*., Br. J. Pharmacol. 151:195, 2007). The P2X7R function was evaluated using 3 mM ATP followed by 2.5 μM ethidium bromide (EB) and analyzed by flow cytometry. Immunocytochemistry and western blotting analysis were used to quantify P2X7R expression. Fixed cells were blocked and incubated with anti-P2X7R primary antibody followed by an anti-rabbit biotinylated antibody and with a fluorophore-conjugated streptavidin. 30 μg proteins were loaded on SDS-PAGE (10%) for western blotting. The membrane was incubated for 1h with non-fat milk (2%) followed by primary antibody anti-P2X7R or monoclonal anti-β-actin and with a peroxidase-conjugated antibody. MECs were loaded with 2.5 μM DAF-FM and used for nitric oxide (NO) measurement induced by 100 μM BzATP, in presence or not of P2X7R antagonists (1 μM KN-62 or 50 nM A740003). The ectonucleotidases activity was measure using MECs stimulated by 50 μM ATP plus 32P-ATP and the radioactivity was quantified by liquid scintillation counting. MECs were treated with 30 μM 2metilSATP (4h), a P2Y1R agonist, and then mononuclear cells obtained from both groups were co-incubated. After 30 min, the wells were washed and photographed using Microscope Olympus IX71. **Results and Discussion:** P2X7R expression was observed in both groups, but the infected mice showed a lower expression than control (78.6 ± 3.2 and 100.4 ± 5.8%, n = 4, $P<0.05$, respectively). Accordingly, MECs from infected mice stimulated with 3 mM ATP showed a lower EB uptake than the controls (25 ± 2%; 43 ± 1%, n = 8 and 10, respectively, $P<0.05$). BzATP induced a higher NO synthesis in control than in infected MECs (22.2 ± 2.1% and 4.8 ± 1.8%, n = 15, respectively, $P<0.05$), and the pre-treatment with a P2X7R antagonist reduced BzATP effect being similar to the NO synthesis in $P2X7R^{-/-}$ group (6.1 ± 1.5%, n = 15). In the infected mice, the P2X7 reduced function was accompanied by an increase of the ectonucleotidases activity (6.9 ± 1.1; n = 16 and 17 ± 3.4 n = 14 pmol Pi/μg of protein, $P<0.05$, control and infected, respectively). The higher ectonucleotidases activity could increase ADP, an agonist of P2Y1R which is related with adhesion of leukocytes to endothelial cells (Zerr *et al*., Circ 123:2404-2413, 2011). In the control group 2metilSATP stimulated cell adhesion ($P<0.05$), but we did not observe a significant effect in the infected group. Our current data suggest that schistosomiasis is related to an increase of ectonucleotidases function possibly reducing high extracellular ATP levels, and this is accompanied by a reduction of P2X7R expression and function, that could limit vascular inflammation during chronic infection. **Financial support:** CNPq, FAPERJ-PRONEX, FAPERJ

04.038 Effect of the antioxidant epigallocatechin-3-gallate in the allergic pulmonary inflammation in lean and obese mice. André DM, Calixto MC, Horimoto CM, Marcondes S, Lopes-Pires ME, Anhê GF, Araújo TMF, Antunes E Unicamp – Farmacologia

Introduction: Obesity increases the prevalence and incidence of asthma. High-fat diet-induced obesity also enhances the pulmonary eosinophilic inflammation and promotes airways and lung parenchyma remodeling in ovalbumin (OVA)-challenged mice. Obesity and asthma are considered chronic inflammatory diseases characterized by increased oxidative stress. We have hypothesized that increased reactive-oxygen species (ROS) and/or reactive-nitrogen species (RNS) in obese mice contribute to aggravate the pulmonary eosinophilic inflammation in allergic animals. The present study aimed to evaluate the effects of epigallocatechin-3-gallate (EGCG), an antioxidant polyphenol flavonoid isolated from green tea, in the pulmonary allergic inflammation in obese and lean mice. **Methods:** The experimental protocols were approved by the Ethics Committee of University of Campinas (UNICAMP; N$^{o.}$ 2469-1). Male C57bl6/J mice received a high-fat diet for 10 weeks. On 8th week, mice were sensitized with OVA (100 µg, s.c). Two weeks thereafter, mice were intranasally challenged with OVA (10 µg). At 48 h after OVA challenge, measurement of cell counts in bronchoalveolar lavage fluid (BALF) and flow cytometry to evaluate ROS production were performed. **Results:** In control (lean) mice, treatment with EGCG (25 mg/kg , i.p, 1h before the first daily challenge) significantly decreased the eosinophil counts ($P<0.05$) compared with untreated mice (0.84±0.11 and 0.53±0.07 x 10^6/ml for untreated and treated mice, respectively). However, EGCG treatment had no effect in eosinophil counts in BALF of obese compared with untreated obese animals. The ROS levels in BALF of OVA-challenged mice were significantly higher than PBS-instilled mice (579±53 and 104 ±18 MIF, respectively), but no significant differences between lean and obese mice were detected. OVA-challenged mice treated with EGCG showed reduced ROS levels in BALF compared with untreated groups, but this reduction was of the same extent in obese and lean mice. **Discussion:** Our present data showed that EGCG decreases eosinophilic infiltration and ROS formation in BALF of lean mice. In obese mice, EGCG decreased ROS formation without affecting the eosinophil infiltration in BALF. It is likely that a dose adjustment of EGCG (higher dose and/or other therapeutic regimen) is required to further evaluate its effect in asthma of obese mice. **Financial support:** CNPq/ FAPESP

04.039 Response inflammatory presents in acute pancreatitis induced by taurolithocholic acid was reverted by fucoidin, **P and L-selectin blocker**. Carvalho ACS[1], Sousa RB[1], Costa JVG[1], Silva LMN[1], Mendes WO[1], Costa MR[1], Franco AX[1], Ribeiro RA[1], Criddle DN[2], Soares PMG[3], Souza MHLP[1] [1]UFC – Fisiologia e Farmacologia, [2]University of Liverpool, [3]UFC – Morfologia

Introduction: Acute pancreatitis is an acute clinical disease, characterized by acute onset, rapid progression, systemic inflammatory response (SIR) and high mortality. Leucocyte accumulation at sites of inflammation is a multistep process controlled by specific adhesion molecules of the selectin and integrin families. These adhesive mechanisms have not been studied in detail in the pancreas. Our aim was to evaluate the effect of the treatment with fucoidin (P and L-selectin blocker) in a model of acute pancreatitis induced by taurolithocholic acid. **Methods:** Swiss male mice (25-30g) were assigned for saline (S), sham (Sh), taurolithocholic (TC) or fucoidin + taurolithocholic (F+TC) groups. Thaurolithocolic acid (TLC-S), 50 µl 2%, or saline was retrogradely infused into the mouse pancreatic duct. In another group, the animals was treated with fucoidin (25 mg/kg i.v.) 30 min before the surgery. The animals were killed 24 hours later and samples of pancreas (P) and lungs (L) were collected for assessment of MPO activity. Plasma (Pl) samples were collected to determine amylase, lipase, nitrite and cytokines concentrations (TNF-α, IL-1β and CXCL-8), by ELISA. All animal procedures were approved by the local ethics committee (protocol 34/10). Significance statistics (tests ANOVA and Bonferroni), values considers with $p<0.05$. **Results:** Fucoidin was able to reverted the increase in plasma amylase (U/l) (S = 3252.9±431.0; Sh = 4280.7±373.6, TC = 6808.4±364.5 and F+TC = 3877.70±519.50) and lipase (U/l) levels (S = 449.20±37.90; Sh = 503.60±89.17, TC = 865±75.90 and F+TC = 488.60±32.71). The increase of the neutrophil infiltrate was also reverted by treatment with fucoidin when observed the MPO activity (UMPO/mg of tissue) (Pancreas: S = 2.39±0.27, Sh = 3.14±0.68, TC = 7.28±0.85, F+TC = 3.37±1.68; Lungs: S = 4.94±0.49, Sh = 5.09±0.43, TC = 7.72±0.86, F+TC = 5.13±0.58). The increase of nitrite (µM) and pro-inflammatory cytokines (pg/ml) in plasma were reverted by treatment with fucoidin (nitrite: S = 7.81±2.36, Sh = 12.52±2.67, TC = 46.18±8.64, F+TC = 22.16±2.97; pro-inflammatory cytokines: TNF-α: S = 80.96±36.36, Sh = 28.91±9.76, TC = 206.90±47.01, F+TC = 42.96±17.67; IL-1β: S = 111.9±3.10, Sh = 121.1±3.95, TC = 168.2±8.60, F+TC = 139.1±3.10 and CXCL-8: S = 449.70±46.07, Sh = 440.70±70.98, TC = 1021±187.60, F+TC = 314±104.20) when compared to the saline groups. **Discussion:** The inflammatory parameters presents in taurolithocholic-induced acute pancreatitis were reverted by treatment with fucoidin. Our results suggest that P and L-selectin is an important pathway in pathophysiology of acute pancreatitis. **Financial support:** CAPES, CNPq and FUNCAP

04.040 Evaluation of periradicular lesions in a rat model of type-2 diabetes: Effects of treatment with the antioxidant tempol. Oliboni PB[1], Zollmann LA[2,3], Wolle CFB[1], Leite CE[2], Campos MM[1,2] [1]FO-PUCRS, [2]PUCRS –Toxicologia e Farmacologia, [3]PUCRS – Farmácia

Aim: Type-2 diabetes has been associated with an increased risk of oral complications, including dental infections (Mindiola et al., J Endod. 32; 828, 2006). This study was designed to evaluate the development of periapical lesions in a rat model of insulin resistance type-2 diabetes, and to assess the therapeutic potential of systemic treatment with the antioxidant agent tempol on apical periodontitis.

Methods and Results: Male Wistar rats (100-120 g; N = 5 per group) were used. All the experimental protocols were approved by the local animal ethics committee (CEUA/09/00132). The rats received tap water (control group) or 20%-glucose solution (type-2 diabetes groups) during nine weeks. Six weeks after the onset of glucose administration, the pulps of the mandibular first molars were surgically exposed with a ¼ size round steel bur in high-speed rotation, under constant irrigation, and they were left open to oral cavity for 21days. During the last three weeks, type-2 diabetic rats received saline solution (0.9 % NaCl; 10 ml/kg) or the antioxidant tempol (50 or 100 mg/kg) by oral route. The animals were checked to register the body weight, as well as the food and drink consumption throughout all the experimental phase, as indicatives of diabetes signals such as polyuria, polydipsia and polyphagia. Following euthanasia, the mandibles have been removed, and the extent of periapical lesions was measured radiographically and compared among the different experimental groups. The rats in the type-2 diabetes group displayed a general elevated gain of body weight, associated to a reduction of food eating and an increase of drink consumption, which were generally reversed in tempol-treated animals. Radiographic data demonstrates that extension of periapical inflammatory lesions was not significantly different according to evaluation of control (7140 ± 432 pixels/cm) versus type-2 diabetic rats (6243 ± 510 pixels/cm). Unexpectedly, the treatment with tempol was not able to significantly alter the apical periodontitis development in diabetic animals, in either doses of 50 or 100 mg/kg (6323 ± 577 and 5799 ± 379 pixels/cm, respectively). Otherwise, the administration of tempol (50 mg/kg) significantly reduced the size of periapical lesions in control rats (52 ± 7 % of reduction). **Conclusion:** Our results provide evidence showing the lack of effectiveness of the antioxidant tempol in rats with insulin-resistance type-2 diabetes, in comparison to control animals. Further studies are in development to assess the effects of anti-diabetic drugs on that condition. **Financial support:** BPA-PUCRS, CNPq, FAPERGS and FINEP/PUCRSINFRA #01.11.0014-00.

04.041 Effect of zingiber essential oil treatment on renal parameters and in the expression of cytokines proinflammatory TNF-α and anti-fibrotic BMP-7 after renal ischemia and reperfusion in mice. Pinho RJ[1], Damião MJ[1], Silva FMS[1], Aguiar RP[1], Yamada AN[1], Freitag AF[1], Giannocco G[2], Duarte JS[2], Oliveira K[2], Cuman RKN[1]
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Introduction: The ischemia (I) is related to the interruption of blood supply in oxygen and nutrients during a determined period of time. In the ischemic injury after renal reperfusion (R) where the blood flow is restored in the ischemic tissue, an increased creatinin is observed. It has been demonstrated many biological activities for this oil, such as: analgesic, anti-inflammatory and immunomodulatory. In this work the effect of Zingiber essential oil (GEO) treatment in the renal function and in the expression of cytokines pro-inflammatory TNF-α and anti-fibrotic (BMP-7) were evaluated in murine experimental models of renal ischemia and reperfusion. **Methods:** The protocol regarding to this study was approved by the ethical commission of ethics in animal research (041/2008/CEAE/UEM). Male Swiss mice (20 to 28g) were anaesthetized with ketamine (100 mg/kg ; *i.p.*) and xylazin (10 mg/kg , *i.p.*). The animals were submitted at the left renal pedicle unilateral I/R procedure during 45 minutes. The experimental groups were: a) GEO (100, 200 or 400 mg/kg , once a day); b) Control: animals submitted to I/R procedure; and c) Sham: non-isquemic animals receiving saline. The animals were treated by *gavage* during 48 hs. The mice were euthanazied and the blood was collected from abdominal aorta in mice under anesthesia, and then centrifugated. The serum was separated for seric creatinin determination by method Jaffé modified. Total RNA was isolated from kidney tissue using Trizol reagent and the amplification was performed using the technique of RT-PCR. The results were expressed as means ± SEM, and were statistically analyzed by ANOVA ($P \leq 0.05$). **Results: serum creatinin**: **Significant differences were observed for creatinin levels after GEO treatment, when compared to sham group in all doses tested: [C*ontrol*: 0.54±0.006mg/dL*; Sham: 0.41±0.001mg/dL; $Dexa_{1\ mg/kg}$: 0.42±0.01*mg/dL; $GEO_{100\ mg/kg}$: 0.41±0.02*mg/dL; $GEO_{200\ mg/kg}$: 0.43±0.10*mg/dL and $GEO_{400\ mg/kg}$: 0.42±0.03mg/dL*] (*P<0.05). A significant inhibition in the expression of TNF-α mRNA was observed in all doses tested, when compared to Control group (*P<0.05): [Control: 1.69±0.10;Sham: 1.00±0.04*; $Dexa_{1\ mg/kg}$:0.77±0.11; $GEO_{100\ mg/kg}$: 0.60±0.05; $GEO_{200\ mg/kg}$:0.32±0.04; and $GEO_{400\ mg/kg}$: 0.32±0.02] and in the BMP7 expression levels, when compared to Control group (*P<0.05): [Sham: 6.16±0.68*; Control: 1.00±0.04; $Dexa_{1\ mg/kg}$: 1.65±0.22*; $GEO_{100\ mg/kg}$: 1.57±0.33*; $GEO_{200\ mg/kg}$:3.70±0.87*; $GEO_{400\ mg/kg}$: 5.89±0.44*]. Conclusion**: The GEO treatment restored the renal function evaluated by serum creatinin levels. On the other hand, since a significant inhibition in the TNF-α mRNA expression levels and an increase in the BMP-7 expression at dose 200 and 400 $_{mg/kg}$ were observed, our data suggest protective effect of GEO in experimental renal ischemia and reperfusion in mice. **Acknowledgments**: CAPES, CNPq, Fundação Araucaria

04.042 Pharmacological evaluation of a new series of sulfonamide derivatives designed as modulators of lung inflammation. Souza ET[1], Carvalho VF[2], Ferreira TP[1], Ciambarella BT[1], Lima LM[2], Barreiro EJ[2], Martins MA[1], Silva PMR[1] [1]IOC – Inflamação, [2]UFRJ – Avaliação e Síntese de Substâncias Bioativas

Introduction: Phosphodiesterase 4 (PDE4) has been proposed as a critical factor in the pathogenesis of inflammation by metabolizing cAMP in different cell types. The design and synthesis of a new series of sulphonamide derivatives planned by structural modification of the prototype LASSBio-448 have been described. LASSBio-448 was shown to be a PDE 4 inhibitor with therapeutic index and potency higher than of the standard inhibitor (R,S)-rolipram. The present study aimed at evaluating the pharmacological profile of a new series of sulfonamide derivatives both *In vitro* and *in vivo* systems. **Methods:** A series of 13 derivatives was first screened for its anti-PDE 4 (PDE4A1A, PDE4B1, PDE4C, PDE4D3) activity by IMAP^{TM} TR-FRET system (Molecular Devices). The standard PDE4 inhibitor rolipram was used as control. The compounds were tested on alveolar macrophage (AMJ2C11 cell lineage) stimulated with LPS (1 ng/mL) and TNF levels was quantified in the 6 h- supernatant by ELISA. For *in vivo* system, female A/J mice were instilled with LPS (25 µg) and the compounds were administered by oral route (25 - 100 µmol/kg), 1 h before stimulation. The analyses were made 24 h after LPS and included: i) lung function (resistance and elastance) and airways hyperreactivity to methacholine (Fine-point Buxco System) and ii) leukocyte infiltration in the bronchoalveolar lavage (BAL). Total and differential leukocyte counts were performed in Neubauer chamber and cytocentrifuged smears coloured with May-Grunwald-Giemsa dye. All experimental procedures were approved by Ethics Committee of Animal Use of FIOCRUZ (License 034/09). **Results:** We showed that at the concentration of 10 µM LASSBio-1612, LASSBio-1628, LASSBio-1631 and LASSBio-1632 had the ability to inhibit the PDE41A and PDE4D3 activity, but not PDE4B1 and PDE4C. The other compounds tested had no effect on PDE4 isoenzymes. Further, incubation of macrophages with the four compounds as well as rolipram (0.1 -10 µM), 1 h before stimulation with LPS, significantly reduced the levels of TNF released. When tested *in vivo*, only LASSBio-1632 inhibited the inflammatory response caused by LPS, including increased lung resistance and elastance as well as airways hyperreactivity to methacholine. Total leukocyte and neutrophil infiltration in the BAL fluid was also suppressed by the compound. Rolipram abolished the LPS-induced inflammation as expected. **Conclusion:** Our results show that screening of a new series of sulfonamide derivatives led us to identify LASSBio-1632 as a compound which had the ability to inhibit: i) PDE4A and PDE4D3 isoenzyme activity, ii) activation of macrophages *In vitro,* and iii) lung acute inflammatory response caused by LPS in mice. Altogether, these data indicate that LASSBio-1632 seems to be a promising anti-inflammatory compound, though additional experiments are needed to clarify better its pharmacological profile. **Financial Support:** FIOCRUZ, CNPq, FAPERJ, INCT-INOFAR, PRONEX 2009.

04.043 Antipyretic effect and central nervous system amount of dipyrone active metabolites, **4-methylaminoantipyrine (4-MAA) and 4-aminoantipyrine (4-AA)**. Malvar DC[1], Aguiar FA[2], Vaz ALL[2], Assis DCR[1], Melo MCC[2], Clososki GC[2], Jabor VAP[2], Souza GEP[2] [1]FMRP-USP – Farmacologia, [2]FCFRP-USP – Física e Química

Introduction: Dipyrone is a potent antipyretic and analgesic prodrug. After administration dipyrone is hydrolyzed to 4-MAA which undergoes metabolism in the liver to 4-AA by demethylation and to 4-formylaminoantipyrine (4-FAA) by oxidation. Subsequently, 4-AA is converted into 4-acetylaminoantipyrine (4-AAA) by acetylation (Zylber-Katz, *Clin Pharmacol Ther*. 58:198, 1995). We recently demonstrated that the cyclooxygenases inhibitors 4-MAA and 4-AA are the active metabolites on LPS-induced fever, while 4-MAA is the active on the PGE_2-independent fever induced by *Tityus serrulatus* venom (Malvar et al., 2012; abstract from 4º PPTR – Búzios/Brazil). We also showed that the antipyretic effect of dipyrone is unrelated to PGE_2 synthesis inhibition in the hypothalamus (Malvar, *Br J Pharmacol*. 162:1401, 2011). Thus, the aim of this study was to evaluate the PGE_2 amount on CSF and hypothalamus after LPS injection and dipyrone metabolites amount on plasma, CSF and hypothalamus in animals treated with i.p. antipyretic doses of dipyrone, 4-MAA and 4-AA. We also investigated the antipyretic effect of intracerebroventricularly (i.c.v.) injected dipyrone, 4-MAA and 4-AA. **Methods:** Male Wistar rats (200g, n = 6-8) received i.c.v. injection of saline, dipyrone, 4-MAA or 4-AA (120-360µg/rat) 30 min before the i.p. injection (0.5 ml) of saline or LPS (50µg kg^{-1}). Body temperature was measured for up 6 h by radiotelemetry. CSF and hypothalamic PGE_2 levels was measured by ELISA in animals (n = 6) treated i.p. with 4-MAA and 4-AA (90mg kg^{-1}) at 3 h after LPS (50µg kg^{-1}, i.p.) injection. Dipyrone metabolites levels on plasma, CSF and hypothalamus were measured by HPLC-MS-MS 1.5, 2.5, 3.5 and 5.5 h after i.p. administration of dipyrone (120mg kg^{-1}), 4-MAA and 4-AA (90mg kg^{-1}). Ethical commission protocol nº 200/2008 – CETEA/FMRP-USP. **Results:** I.p. administration of 4-MAA (90mg kg^{-1}) or 4-AA (90mg kg^{-1}) inhibited the fever (from 38.6±0.09 to 37.1±0.12 and 37.0±0.09, respectively) and the PGE_2 increase on CSF (from 1189±140.7 to 50.2±16.2 and 33.8±10.5 pg ml^{-1}, respectively) and hypothalamus (from 1031±74.7 to 381.1±39.4 and 289.2±73.7 pg g^{-1} of tissue, respectively) after i.p. LPS injection. All dipyrone metabolites were found on plasma, CSF and hypothalamus after i.p. dipyrone or 4-MAA administration, while only 4-AA and 4-AAA were found in these sites after i.p. 4-AA administration. Dipyrone (120mg kg^{-1}, i.p.) treated animals showed less bioavailability of all dipyrone metabolites when compared to 4-MAA (90mg kg^{-1}, i.p.) or 4-AA (90mg kg^{-1}, i.p.) treated animals. Finally, i.c.v. dipyrone (120-360µg/rat), 4-MAA (120-360µg/rat) or 4-AA (120-360µg/rat) administration did not change the body temperature of animals after either LPS or saline. **Conclusion:** These results demonstrated that the i.p. antipyretic dose of 4-MAA and 4-AA (90mg kg^{-1}) promoted more plasmatic, CSF and hypothalamic bioavailability of all dipyrone metabolites than dipyrone (120mg kg^{-1}), which may explain why 4-MAA and 4-AA, but not dipyrone, inhibited hypothalamic PGE_2 concentration at i.p. antipyretic dose. Moreover, our results also suggest that the antipyretic effect of dipyrone, 4-MAA and 4-AA did not involve central mechanisms. However, more studies are necessary to confirm this hypothesis. **Financial support:** FAPESP (2008/09443-4).

04.044 Dysregulation of the inflammatory response in pneumosepsis by early lipoxin A4 production. Sordi R[1], Menezes-Lima-Júnior O[2], Horewicz V[3], Scheschowitsch K[1], Santos LF[1], Assreuy J[1] [1]UFSC – Pharmacology, [2]Fiocruz, [3]UFSC – Microbiology, Immunology and Parasitology

Objective: Pneumonia is the major cause of sepsis in intensive care units. Sepsis is the result of a systemic inflammatory response associated with a microbial infection. While activating a pro-inflammatory component, sepsis also induces an anti-inflammatory response. Imbalance of these two components may lead to the inflammatory dysfunction seen in sepsis. Lipoxin A_4 (LXA_4) is an endogenous lipid mediator with potent anti-inflammatory and pro-resolutive actions and binds mainly to FPR2/ALX receptors. The aim of this work was to investigate the participation of LXA_4 and FPR2/ALX receptor in sepsis and their role in the dysregulation of inflammatory process that occurs in this pathology. **Methods:** Pneumosepsis was induced by intra-tracheal inoculation of *Klebsiella pneumoniae* in mice. Sham-inoculated mice were used as control. A group received the selective FPR2/ALX antagonist BOC-1 (1 µg/kg) one hour after inoculation. Six and 24 hours after sepsis induction, animals were killed and bronchoalveolar lavage (BAL) and tissues were obtained for analysis. We evaluated LXA_4 biosynthesis in sepsis, FPR2/ALX expression in BAL cells by flow cytometry and in lung tissues by immunofluorescence, and the effects of BOC-1 in cytokine production, cell migration, bacterial load and survival rate. **Results:** Septic animals exhibited a high degree of bacterial load in BAL, spleen and heart, increases in pro- and anti-inflammatory cytokines, increased leukocyte migration into the focus and a mortality rate about 50%. Moreover, septic animals exhibited an increase in FPR2/ALX receptor expression in lung tissue and BAL cells, mainly in immature CD11b+ Gr-1+ myeloid cells. Interestingly, LXA_4 levels in the infectious focus increase in the first 6 hours after inoculation. When septic animals were treated with the FPR2/ALX antagonist BOC-1, they exhibited increased neutrophils and immature myeloid cells in the infectious focus and consequently a reduced bacterial load and dissemination. BOC-1 treatment also increased IL-1beta levels in infectious focus. Finally, treatment with BOC-1 increased survival rate. **Conclusions:** Anti-inflammatory LXA_4 is increased in the early phase of sepsis. The anti-inflammatory effect of LXA_4 may contribute to the inflammatory dysregulation that occurs in this pathology, because an infection process is going on and needs to be controlled. Therefore, this early production of LXA_4 together with the increased expression of its receptor may favor the spread of bacteria and the high mortality. The reduction in LXA_4 anti-inflammatory mechanisms in the beginning of the pneumonia-induced sepsis may be an interesting therapeutic alternative. **Financial support**: CNPq, CAPES, FAPESC, FINEP.

04.045 Nocturnal melatonin priming endothelial cells by the modulation of NF-kB activation. Marçola M, Tamura EK, Markus RP [1]IB-USP – Fisiologia

Introduction: Endothelial cells constitute the vascular internal layer and are responsible for vascular homeostasis and several aspects of the inflammatory response. Reduction in nocturnal plasma melatonin due to the administration of lipopolysaccharide (LPS) primes endothelial cells, as the expression of adhesion molecules in primary endothelial cells culture inversely correlates with the plasma melatonin concentration (Tamura, PlosOne. 5:1, 2010). On the other hand, the reduction of the endothelial cells to a quiescent state regarding the expression of inducible nitric oxide synthase and adhesion molecule expression (Tamura, J Pineal Res. 46:268, 2009, Sasaki, BMC. Gastroenterol., 24:2, 2002) due to the inhibition of the nuclear factor kappa B (NF-kB) activation in cultured cells, requires concentrations of melatonin much higher than those found in nocturnal plasma. Thus, we aimed to verify whether the daily variation of melatonin could also prime endothelial cells regarding the nuclear translocation of NF-kB in cells cultured for at least 20 days. **Methods:** Endothelial cells obtained from cremaster muscle of rats killed during daytime (six hours after lights on) or nighttime (six hours after lights off) were cultivated for at least 20 days, till confluence. NF-kB nuclear content and subunits were determined by gel shift and super-shift assays, respectively. Specific antibodies for p50, RELA, p52, c-REL, RELB and BCL3 were purchased from Santa Cruz Biotechnology. Plasma melatonin was quantified by ELISA (IBL). All animal procedures were performed under the ethical conditions of the Institute of Bioscience of University of São Paulo (license 124/2011). **Results:** Two-distinct NF-kB complexes were detected in all samples, and selective antibodies suggested the presence of p50/p50 and p50/RELA dimers. The hour of the day that the animal were killed significantly interferes in NF-kB activation, its content is reduced to 36.7 ±14.3 % (n = 10) when nighttime was compared to daytime group. The most interesting result in the study is that the amount of NF-kB translocated to the nuclei is inversely related to the plasma concentration of melatonin (Person's $r = -0.80$; $p<0.05$), strongly suggesting that the daily variation of melatonin is important for controlling endothelial cells activation state. **Discussion:** This study, together with other data of our group, indicates that the daily variation of melatonin is an important factor to controlling innate immune response, as the ability of endothelial cells to activate the hallmark nuclear factor for inducing proteins that play roles in the mounting of innate immune responses, is directly modulated by melatonin. Another important conclusion is that melatonin primes endothelial cells to a more quiescent state, and the absence of this hormone in culture is not enough to turns these cells to the most active state observed during daytime. Therefore, this study opens a new way to investigate the daily modulation of endothelial cells activity. **Finnancial Support**: FAPESP (07/07871-6; 2011/01304-8), CAPES and CNPq (472881/2009-4).

04.046 Acute pancreatitis induced by caerulein causes important alterations inflammatory and functional in lung of the rat. Morais CM[1], Silva LMN[1], Mendes WO[1], Costa MR[1], Xavier AF[1], Souza EP[2], Ribeiro RA[1], Souza MHLP[1], Criddle DN[3], Soares PMG[2] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Morfologia, [3]University of Liverpool

Introduction: Acute pancreatitis is an acute clinical disease, characterized by rapid progression, systemic inflammatory response (SIR) and high mortality. Lung injury is a severe complication of acute pancreatitis that increases the mortality rate. Our aim was to evaluate the inflammatory and functional changes of the lung in the course of experimental acute pancreatitis induced by caerulein. **Methods:** Male Wistar rats (80-150g) were treated four times with one hour interval, intraperitoneally with caerulein(C) (20 µg / kg, suspended in saline) or saline(S). Twenty-four hours after the first injection of caerulein, the animals were anesthetized, tracheostomized and placed in a spirometer for small animals (AD Instruments), with following parameters evaluated: Flow(F), Volume(V), Respiratory Frequency(RF), Raw Tidal Volume(RTV), Tidal Volume(TV) and Minute Volume(MV). Another group of animals were killed 24 hours later and samples of pancreas (P) and lungs (L) were collected for histological analyses and assessment of MPO activity. Plasmatic amylase was measured. All animal procedures were approved by the local ethics committee (protocol 88/11). Values were considered significant with $p<0.05$, using ANOVA and Bonferroni tests. **Results:** Caerulein induced important damage confirmed by histopathological scores in the pancreas (S = 4.66±0.76, C = 7.16±0.65) and lung (S = 6.11± 0.69, C = 8.37±0.32). This damage was dependent on neutrophil infiltration, and MPO activity in pancreas (S = 1.96±0.85, C = 13.04±3.24) and lung (S = 3.54±,0.88, C = 12.4±3.51). Elevated levels of amylase are found in plasma(S = 2897±293.5, C = 5863±732.8). Caerulein also induced significant changes in lung function, F (S = 20.32±1.68, C = 14.40±0.60), V(S = 2.71±0.31, C = 1.71±0.06), RF (S = 136.8±6.50, C = 122.3±2.80), RTV (S = 0.62±0.02, C = 0.54 ±0.02), TV (0.82±0.03, C = 0.70±0.02) MV(S = 111.3±5.6, C = 87.01±3.9). **Conclusion:** Acute pancreatitis induced by caerulein shows inflammatory changes, which correlate with important functional pulmonary alterations. **Financial support:** CNPq and FUNCAP

04.047 Evidence that adenosine and inosine acts in sinergism to exert its anti-inflammatory effects in acute pleural inflammation. Lapa FR[1], Araújo G[2], Buss ZS[3], Fröde TS[3], Cabrini DA[1], Santos ARS[4] [1]UFPR – Farmacologia, [2]UFRN – Ciências Farmacêuticas, [3]UFSC – Ciências Farmacêuticas, [4]UFSC – Ciências Fisiológicas

Introduction: The literature has shown that adenosine and inosine participates in the regulation of inflammation and immune responses, an effect related to activation of adenosine receptors. In agreement, we have previously demonstrated that adenosine and inosine reduced significantly neutrophils migration, pleural leakage and the levels of pro-inflammatory cytokines, in pleural exudates, with involvement of A_{2A} and A_{2B} adenosine receptors (Lapa et al., *Purinergic signal.*, in press, 2012). When adenosine is generated in inflammatory sites, is quickly converted into inosine, by the adenosine deaminase (ADA) enzyme, what led us to hypothetize that adenosine effects were instead, the inosine effects. Thus, the aim of this study was to investigate the metabolism of both purines in acute model of carrageenan-induced pleurisy. **Methods:** Swiss female mice (18-25 g) were treated intraperitoneally with adenosine (0.3, 10 or 100 mg/kg) or inosine (1.0 mg/kg), or pre-treated with EHNA (5 mg/kg), an adenosine deaminase inhibitor, 30 min prior adenosine or inosine. The pleurisy was induced by intrapleural injection of carrageenan (1%) 30 min after treatments, and 4 h later the exudates were collected to determinate total and differential leukocyte counting, adenosine deaminase activity or HPLC analysis. In addition, a number of 6-8 animals were used per group. This study was approved by Ethics Committee of the Universidade Federal do Paraná with protocol no. 320. **Results:** The results showed that the combined treatment with sub effective dose of adenosine (0.3 mg/kg) and inosine (1.0 mg/kg) reduced significantly the total leukocyte and the neutrophil count in exudates, with inhibitions of 56 ± 6 % and 56 ± 4 %, respectively, suggesting a synergistic anti-inflammatory effect. Taking account these results, we evaluate adenosine conversion into inosine, by treating mice with EHNA. The EHNA treatment inhibited ADA activity by 61 ± 1 %. The pre-treatment of adenosine treated mice (10 mg/kg), with EHNA, did not interfere with adenosine anti-inflammatory activity, suggesting that inosine did not contribute with it. Moreover, the HPLC analysis of plasmatic adenosine and inosine levels, of adenosine treated (100 mg/kg) mice, revealed that the concentration of both purines rose together during pleural inflammation peaking at 1 h after induction of pleurisy, with plasmatic concentrations of 0.72 and 0.56 pM, for adenosine and inosine, respectively. Interestingly, was observed that xanthine peaked 30 min., reaching the concentration of 6.23 pM, after carrageenan injection. The levels of uric acid also remained elevated until the fourth hour of pleural inflammation with concentrations of 13.8; 13.9; 8.3; 7.7 pM, at 30 min., 1, 2 and 4 h, respectively. **Discussion and conclusion:** These results revealed us that the anti-inflammatory effects of adenosine seems not to be dependent on its switch on inosine, reinforcing the notion that adenosine and inosine can act together to modulate acute pleural inflammation, strengthening our hypothesis of synergism. Furthermore, our results suggest that xanthine and uric acid can contribute with adenosine and inosine anti-inflammatory effects to control acute pleural inflammation. **Financial Agencies**: REUNI, CAPES, CNPq and FAPESC.

04.048 Hypertension favors the inflammatory process in rats with experimentally induced periodontitis. do-Amaral CCF, Bonato CF, Belini L, Oliveira SHP FOA-Unesp-Araçatuba – Basic Sciences

Objectives: The aim of this study was to analyze bone loss level, neutrophil migration, CXCL2/CINC-2, CXCL5/LIX, CCL20/MIP-3α and TNF-α production, iNOS expression and C-reactive protein (CRP) release in spontaneously hypertensive rats (SHR) and normotensive rats (WTK) submitted to experimental-induced periodontitis (PD). **Methods:** Forty rats were divided into 4 groups: WTK without PD, WTK with PD, SHR without PD and SHR with PD. Periodontitis induction was performed using silk yarn ligatures around the first mandibular molars counterparts. After 14 days, the animals were killed by halothane inhalation. The peripheral blood was collected and CRP, CCL20/MIP-3α and CXCL5/LIX were evaluated by enzyme linked immune sorbent assay (ELISA). In the gingival tissue, bone loss level was determinated histometrically. Neutrophil counts were analyzed by counting the number of neutrophils surrounding the area between the interradicular bone crest and furcation roof in 10 high-power fields (X1000). The level of myeloperoxidase, CXCL2, CXCL5, CCL20 and TNF-α production was measured using ELISA. The iNOS expression was evaluated by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). Data were statistically analyzed using ANOVA with Tukey correlation test ($p<0.05$). **Results:** SHR with PD showed a significant neutrophil recruitment to the inflamed tissue confirmed by the high level of myeloperoxidase when compared to WTK with PD ($p<0.001$). SHR and WTK submitted to periodontitis showed an increased level of TNF-α ($p<0.05$). The production of CXCL2, CXCL5 and CCL20 is increased in SHR animals with or without PD ($p<0.05$). After the induction of periodontal disease CXCL2 and CXCL5 level are increased, but not CCL20 ($p<0.05$). The levels of CCL20 and CXCL5 were increased in the peripheral plasma in SHR without and after PD, but CXCL2/CINC-2 was not detected ($p<0.001$). SHR with PD animals express iNOS mRNA more intensity when compared with WTK with PD ($p<0.001$). C-reactive protein level is increased in animals with PD, and its concentration in SHR with PD was increased when compared to WTK animals ($p<0.05$). The mean of periodontal ligament or bone loss areas of WTK without PD, WTK with PD, SHR without PD and SHR with PD were 0.081±0.012 mm², 0.842±0.138 mm², 0.124±0.024 mm² and 1.513±0.519 mm², respectively, suggesting the bone loss level was more significant in SHR with PD ($p<0.05$). Following the bone loss level we also observed an intense neutrophil recruitment into the interradicular bone crest and furcation roof in SHR and WKT animals with PD ($p<0.001$). The SHR without PD shows a slight increase of neutrophil migration when compared with WKT ($p<0.001$). The amount of lymphocyte, macrophage and eosinophils in the site of inflammation was insignificant. **Conclusions**: The hypertensive state of the genetic SHR seems to favor the inflammatory process, measured using markers such as CXCL2, CXCL5, CCL20, TNF-α, iNOS and CRP, with greater intensity than in normotensive rats. Nevertheless, the periodontitis potentiated the inflammatory process in SHRs. **Financial support**: FAPESP.

04.049 Mechanisms involved on increased nitric oxide synthesis in platelets of lipopolysaccahride-treated rats. Lopes-Pires MA, Naime ACA, Anhê GF, Antunes E, Mendes CB, Marcondes S Unicamp – Farmacologia

Introduction: The effects of lipopolysaccharide (LPS) on platelet activity are quite conflicting and there is only few studies investigating the *in vivo* effects of LPS on platelet reactivity. Previously, our group demonstrated that the treatment of rats with LPS increased intraplatelet reactive oxygen species (ROS) production but this effect did not contribute to the inhibitory effect of LPS on platelet aggregation. In the present work we decided investigate the mechanisms involved in modulating the nitric oxide synthase activity in platelets of LPS-treated rats. **Methods:** The present study was approved by the Committee for Ethics in Animal Research (State University of Campinas – UNICAMP, protocol number 2097-1). Wistar rats (250-320 g) were injected i.p. with saline or LPS (from *E. coli*, 1 mg/kg) and after 6 or 48h arterial blood was collected in ACD-C (9:1 v/v). Cyclic GMP levels were measured using an enzyme immunoassay kit. Nitrated proteins and Akt activation were analyzed by immunobloting. **Results:** Activation of platelets of saline-injected rats with ADP (10 μM) caused a tinny production of cGMP (2,4+0,1 pmol/ml) when compared to the platelets of LPS-treated rats at 6h and 48h (11,4 +0,7 and 8 +1,6 pmol/ml, respectively; n = 3-5). In addition, western blotting analysis showed that protein nitration was increased 3-fold in activated platelets of LPS-treated at 6h and 48h. Incubation of platelets for 10 min with the PKC inhibitor, GF 109203X 10 μM, increased the cGMP levels in control (4-fold) and in platelets 48h after LPS-treatment (1.7-fold). In contrast, in platelets of LPS-treated rats at 6h, the inhibition of PKC abolished the increased cGMP production. The activation of Akt was determinate by analysis of Thr 308 residue phosphorylation. Western blotting assays showed a significant increase of Akt activation in platelets of LPS-treated rats compared to saline-injected animals (4.4- and 3.8-fold increase at 6h and 48h, respectively). **Conclusion:** Our results showed that the treatment of rats with LPS increases NO synthesis in platelets and the formation of cGMP is modulated by PKC dependently on exposition time of animals to LPS. The increase of protein nitration in platelets of LPS treated rats is caused by augmented NO and ROS production (Lopes-Pires et al., 2012). Finally, our results indicated that increase of cGMP level, via PKG, might activate Akt, since previous results of our group showed that inhibition of PI3K does not reduce Akt phosphorilation, which could activate eNOS. These effects constitute a loop of Akt and eNOS activation, resulting in sustained NO production.

References: Lopes-Pires et al., Platelets, 23:195; 2012. **Supported by**: FAPESP

04.050 Hydrogen sulfide donors and their therapeutic potential as antipruritics and anti-inflammatory in mouse dorsal skin. Rodrigues L, Ekundi-Valentim E, Florenzano J, Teixeira SA, Muscará MN, Costa SKP USP – Farmacologia

Introduction: Pruritus (itch) is a sensory modality that, similar to pain, acts as a protective mechanism for the organism. Despite advances in anti-pruritic therapy with histamine antagonists, these agents are ineffective in most patients. Recently, the gaseous mediator H_2S has been highlighted as an important modulator of inflammatory and nociceptive processing mechanisms, but its role in pruritus is unknown. This study was carried out to investigate the effects of H_2S donors Na_2S and Lawesson's Reagent (LR) in the pruritus and skin inflammation evoked by histamine and compound 48/80 (C48/80) intradermally (i.d.) injected in mouse dorsal skin. We have also evaluated the kinetics of H_2S generation in the naïve skin and its enzyme expression; cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE). **Methods:** Under approval of the Institute of Biomedical Sciences Ethics Committee of University of São Paulo (no. 33, book 2/2010), male BALB/c mice (25-30 g) were anaesthetised with isoflurane, and either compound 48/80 (C48/80; 30 µg/site) or histamine (1 µmol/site) alone or in addition to H_2S donors, Na_2S (1-100 nmol/site) and LR (3-300 nmol/site), was intradermally (i.d.) administered in a single (0.05 ml) injection to produce itching, that was measured as a bout of scratching during 40 min. Skin plasma protein extravasation and neutrophil accumulation was assessed in a separate set of animals by the extravascular accumulation of i.v. injected ^{125}I-albumin and increased myeloperoxidase (MPO) activity in the skin, respectively. Data are presented as mean ± SEM. Stats were performed by ANOVA followed by Dunnett's test. $P<0.05$ was taken as significant. **Results:** Either C48/80 or histamine significantly increased itching frequency, and lead to a potent ($P<0.01$) plasma extravasation when compared to its vehicle Tyrode. The pruritus evoked by histamine, was significantly inhibited by 67-77 % with the co-injection of Na_2S (1-3 nmol/site; n = 5-9) and by 47-63 % following co-injection of LR (3-30 nmol/site; n = 6). Moreover the pruritus induced by C48/80 was significantly inhibited by 53-59 % with the co-injection of Na_2S (1-10 nmol/site; n = 5-6). Either co-injection of Na_2S (10–100 nmol/site; n = 5-6) or LR (10–300 nmol/site; n = 5-8) significantly, but not dose-dependently, reduced plasma extravasation induced by C48/80 and histamine or decreased MPO activity evoked by C48/80 in the mouse skin. Western blot analyzes revealed, for the first time, that CSE and CBS are constitutively expressed in murine naïve skin in parallel with basal production of H_2S. **Discussion**: We show for the first time that increasing bioavailability of H_2S in the murine skin, by local application of H_2S donors, exerts a protective role against pruritus and skin inflammation evoked by histamine and C48/80. We suggest that H_2S donors might represent a potential therapeutic class for treatment of pruritus associated with skin inflammation. We also show that there has been a regular production of H_2S in naïve skin along with constitutive expression of CSE and CBS. **Acknowledgements**: Fapesp, Capes and CNPq for **Financial support**. Barreto MAA & Gouveia IM for technical assistance.

04.051 LASSBio-897, **a new *N*-acylhydrazone derivative**, **prevents house dust mite-induced lung inflammation and airways remodeling in a murine model of asthma**. Dalzy DV[1], Cardozo SVS[1], Anjos-Valotta EA[1], Barreiro EJ[2], Fraga CAM[2], Silva PMR[1], Martins MA[1] [1]IOC-Fiocruz – Inflammation, [2]UFRJ – Synthesis and Evaluation of Bioactive Substances – Pharmacy

Introduction: Asthma is a chronic eosinophilic inflammatory disorder of the airways, driven by T lymphocytes and mast cells, which lead to bronchoconstriction, mucus exacerbation and lung tissue remodeling. Inhaled glucocorticoid is a highly effective therapy against asthma, but the refractoriness of some patients and adverse effects limit the benefits of this treatment. The current study was undertaken in order to explore the putative anti-asthma properties of LASSBio-897, a new bioactive compound of the *N*-acylhydrazone class of anti-inflammatory compounds, synthetized from the Brazilian natural substance safrole. **Methods:** Separate groups of A/J male mice were exposed to HDM (*Dermathophagoids pteronyssinus* extract) (15 µg/25 µl, intranasal instillation) or PBS for 3 consecutive days followed by 4 days of rest for 3 weeks. Oral treatment with LASSBio-897 (5 mg/kg) or dexamethasone (1 mg/kg) or vehicle was performed 1 h before each HDM provocation, and the analyses were carried out 24 h after the last HDM or PBS challenge. Lung function, leukocyte infiltration, eotaxin-1 and eotaxin-2 generation, mucus production and peribronchial fibrosis were evaluated. All the procedures involving care and use of laboratory animals were approved by the Animal Ethics Committee of the Fiocruz (n° LW23/10). **Results:** Our findings revealed that HDM-exposed mice mounted robust lung eosinophilic inflammation and airway hyper-reactivity, accompanied by mucus exacerbation and subepithelial collagen deposition. All these changes were clearly sensitive to dexamethasone systemic administration as expected. Similar to dexamethasone, LASSBio-897 treatment almost abolished HDM-induced changes in airway hyperresponsiveness, extracellular matrix deposition, goblet cell hyperplasia and mucus production. The steroid was more effective than LASSBio-897 in inhibiting the numbers of total leukocytes (81% versus 57%), mononuclear cells (48% versus 35%), neutrophils (92% versus 55%) and eosinophils (91% versus 50%) in the BAL fluid, but not in the lung digest samples. Actually, concerning the cellularity in lung tissue, dexamethasone and LASSBio-897 were equieffective in the blockade of eosinophils 45% versus 53%, respectively, but LASSBio-897 was more active than dexamethasone in reducing neutrophil numbers (45% versus 77%, respectively). Finally, dexamethasone abolished both eotaxin-1 and eotaxin-2 production triggered by HDM, whereas LASSBio-897 only inhibited eotaxin-2 (49%). **Conclusion:** Collectively, these data suggest that LASSBio-897 can prevent crucial pathological features of HDM-induced asthma, and should be further investigated as a putative prototype compound in asthma therapy. **Financial support:** CAPES, PRONEX AND INCT-INOFAR.

04.052 Acute effects of estradiol on lung and gut inflammation due to intestinal ischemic insult in male rats. Breithaupt-Faloppa AC[1], Fantozzi ET[2], Romero DC[2], Rodrigues AS[2], Domingos HV[2], Oliveira-Filho RM[2], Vargaftig BB[2], Tavares-de-Lima W[2]
[1]HC-FMUSP, [2]ICB-USP – Farmacologia

Introduction: The intestinal ischemia/reperfusion (i-I/R) causes local and remote injuries that are multifactorial and essentially inflammatory in nature. Sexual dimorphism modulates the profile of Th1 and Th2 lymphocytes, and accordingly sex hormones may modulate the magnitude of acute lung injury (ALI) after i-I/R. Studies indicate that female rats are relatively resistant to organ injury caused by hemorrhagic shock and that gut of female is more resistant than the male to deleterious effects of ischemic injury. Here we investigated the effect of estradiol treatment in male rats on the lung and intestinal inflammation after i-I/R. **Methods:** Anesthetized male Wistar rats were subjected to occlusion of the superior mesenteric artery (SMA) during 45 min, followed by 2 hr of reperfusion. Sham-operated rats were used as controls. An additional group of non-manipulated rats was added to obtain normal values of the variables studied (Basal). Groups of rats were treated with estradiol (E2) (17βestradiol, 280ml/kg, s.c. 24 hours before ichemia, or i.v. 30 min after induction of ischemia). Lung and intestinal vascular permeability (LVP and IVP) was assessed by the Evans blue dye extravasation method. Neutrophil recruitment to the tissues was assessed by the standard myeloperoxidase (MPO) method. Circulating neutrophils obtained from rats after reperfusion were maintained in direct contact with a human umbilical vein endothelial cells (HUVEC) monolayer for 4 hr. The HUVEC detachment was evaluated by comparing the optical density of the HUVEC layer after experiments with a confluent monolayer. **Results:** Rats submitted to i-I/R presented increased LVP (sham = 63.7±8.2; I/R = 192±15.4; n = 5) and IVP (sham = 72.9±9.5; I/R = 113.9±7.4; n = 5) as compared to sham-operated controls. MPO activity was found elevated in the lungs (sham = 0.78±0,02; I/R = 2,91±0,08; n = 5) and gut after i-I/R (sham = 0.19±0.02; I/R = 0.76±0.04; n = 5). Both protocols of estradiol treatment led to reduction of LVP (24 hr = 135.4±20.2; n = 5). Regarding to IVP, estradiol treatment 24 h before i-I/R had no effect, but when given after 30 min of ischemia led to an increase of IVP (30 min = 174.4±20.9; n = 5). MPO activity in the lungs and gut was reduced only by the estradiol treatment after 30 min of ischemia (lung = 1.6±0.15; gut = 0.36±0.05; n = 5). The HUVEC detachment (I/R = 5.7±1.1; n = 10) caused by circulating neutrophils obtained after reperfusion were also reduced when the rats received estradiol 24 hr before i-I/R (24 hr = 0.97±0.4; n = 10). **Conclusion**: Estradiol treatment is able to reduce lung and gut inflammation due to intestinal ischemia/reperfusion and alter the neutrophil capability of reducing the integrity of endothelial layer *In vitro*, suggesting that estradiol might play a role on neutrophil activity and recruitment and microvascular permeability after intestinal trauma. Ethics committee: nº 080, page #019, book #02. Supported by CNPq and FAPESP.

04.053 Role of CCR2 in neutrophil articular infiltration in arthritis. Talbot J[1], Bianchini FJ[1], Souto FOS[2], Nascimento DCB[1], Pinto LG[1], Peres RS[2], Oliveira RD[3], Almeida SL[3], Silva JR[2], Ferreira SH[1], Louzada-Junior P[3], Cunha TM[1], Cunha FQ[1], Alves-Filho JC[1] [1]FMRP-USP – Farmacologia, [2]FMRP-USP – Imunologia, [3]HC-FMRP-USP – Clínica Médica

Background: Rheumatoid Arthritis (RA) is an autoimmune arthropathy characterized by joints pain, synovial hyperplasia and progressive damage of cartilage and bone joint. These features of arthritis have been associated with neutrophil infiltration on articular cavity. Blood neutrophils (PMN) trafficking during inflammation is a complex process which several types of chemotactic factors which may include lipids, complement activation products and especially CXC chemokines. In general, CXC chemokines, MIP-2 and KC, appear to be involved in mediating PMN influx into tissues, while CC chemokines (CCLs) interact predominantly with monocytes and lymphocytes. However, our group demonstrated that blood neutrophils from mice and patients with sepsis express de novo the CC chemokine receptor type 2 (CCR2). Even, it was demonstrated that CCR2 expression has an essential role in neutrophil tissue infiltration and multiple organ dysfunction in sepsis. Previously, we found high levels of CCR2 ligand (CCL2) in synovial fluid of Rheumatoid Arthritis Patients. Here, we investigate the expression of CCR2 on neutrophils from Rheumatoid Arthritis Patients and the role of CCR2 expression in neutrophils in a mouse model of experimental arthritis. **Methods:** Neutrophils from healthy or Rheumatoid Arthritis Patients were purified and used to chemotaxis assay and PCR evaluation of CCR expression. The same was done for neutrophils from experimental arthritis. WT and CCR2 deficient mice (CCR2-/-) were sensitized (s.c.) with 500 ug of mBSA in a emulsion containing complete Freund´s adjuvant. Booster injections of mBSA in incomplete Freund´s adjuvant were given 7 and 14 days after the first injection. Arthritis was induced in the immunized mice 21 days after the initial injection by intra-articular (i.a.) injection of mBSA (10 µg/cavity) or MCP-1 (50ng/cavity). Neutrophil migration was assessed 6hs after i.a. challenges. Articular hypernociception was evaluated at this same time using an electronic version of the von Frey test. Cytokine determination in i.a lavage was realized by ELISA. In some experiments, mice were treated with CCR2 antagonist (30 mg/kg , RS504393) i.v. twice, 24 h and 1 h before the challenges. *In vitro* neutrophil chemotaxis in response to MIP-2 (30ng/ml) or MCP-1 (1ng/ml) was performed using a Boyden microchamber. CCR2 expression in neutrophils isolated from blood was determined by PCR-Real Time (m RNA) and immunofluorescence. All experiments were approved by HCFMRP/USP Human Ethics Committee (2981/2009) and Animal Ethics Committee from FMRP/USP (nº 181/2008). **Results:** Results show that human and murine neutrophils isolated from blood of arthritic mice express CCR2 and respond *In vitro* to CCL2 chemokine. Even more, neutrophils from bone marrow of arthritic mice do express a functional CCR2. Furthermore, CCL2 was able to induce neutrophil intra-articular infiltration in immunized mice. Neutrophil recruitment to joints and articular hyperalgesia induced by antigen challenge (mBSA) was significantly reduced in CCR2-/- mice or by treatment with CCR2 antagonist. To ensure that CCR2 were directly responsible of neutrophil infiltration to joint cavity in arthritis we performed the adoptive transference of WT neutrophils from immunized mice to CCR2-/- immunized mice. This transference restored arthritis phenotype (neutrophil infiltration and articular hypernociception) to CCR2-/- mice after mBSA challenge. **Conclusion:** Our findings suggest that CCL2-CCR2 chemotaxis pathway is involved directly in the detrimental infiltration of neutrophils to the joints in experimental arthritis. Therefore, we envisage that CCR2 blockage is a potential strategy for human rheumatoid arthritis treatment.

04.054 Protein fraction of *Calotropis procera latex* reduces mechanical hypernociception in mice: **Involvement of NO and KATP channels**. Carmo LD[1], Luz PB[1], Pinheiro RSP[1], Freitas LBN[1], Aragão KS[1], Bitencourt FS[1], Alencar RN[1], Alencar NMN[1], Ramos MV[2] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Bioquímica e Biologia Molecular

Background and aims: *Calotropis procera* is a shrub found in tropical Asia, Africa and South America, which has been used in traditional medicine for the treatment of various diseases. This plant produces milky latex with anti-inflammatory and analgesic effects, demonstrated in different experimental models. We aim here to evaluate the effects of a protein fraction isolated from the latex of *Calotropis procera* (LP) on mechanical hypernociception induced by prostaglandin E_2 (PGE_2). **Methods:** a) Swiss male mice were used (n = 5, 25 - 30 g). b) Mechanical hypernociception (MH) was evaluated using the electronic version of the von Frey before (T0) and 3h after the injection of PGE_2 into the mice's paw (100ng/paw). LP (5 and 50 mg/kg , i.v.) was administered 30 minutes before PGE_2. The animals were evaluated after 3 hours. c) To assess the involvement of NO, it was administered L-arginine, (400 mg/kg , i.p.), an amino acid precursor of NO; L-NAME (30 mg/kg , i.p.), a non-selective inhibitor of NO synthase, or LP (5 mg/kg , i.v.) 30 minutes before PGE_2. d) To evaluate the role of KATP channels, it was administered glibenclamide (5 mg/kg i.p.), KATP channels blockers; diazoxide (3 mg/kg i.p.), involved in opening the K_{ATP} channels, or LP (5 mg/kg i.v.) 30 minutes before PGE2. e) For statistical analyzes we used the ANOVA / Bonferroni, $p<0.05$ was accepted. f) This study was approved by the Institutional Ethics Committee (61/11). **Results:** LP (5 and 50 mg/kg i.v.) inhibited the MH induced by PGE_2 in 42% and 40%, respectively. L-NAME administered with LP (5 mg/kg i.v.) showed inhibition of 18%. L-NAME reversed the antinociceptive effect of latex in HM induced by PGE_2. LP (5 mg/kg i.v.) administered with glibencamide decreased HM by 3% when compared to PGE_2. Glibenclamide reversed the beneficial effect of latex in HM. **Conclusion:** The results indicate that LP shows a potent antinociceptive effect on mechanical hypernociception evoked by PGE_2. This effect may be associated with involvement of NO and KATP channels. **Financial support:** CAPES and CNPq.

04.055 Role of integrin alphaDß2 in the early phase of pulmonary inflammation caused by silica particles in mice. Ferreira T[1], Carvalho V[1], Arantes ACS[1], Zimmerman G[2], Abreu A[3], Cordeiro RSB[1], Martins MA[1], Faria-Neto H[3], Silva P[1]
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Introduction: Integrins are plasma membrane heterodimers that are broadly distributed on different cell types and mediate critical functions, including adhesion, homing, signaling, and gene expression. Leukocyte integrins are required for host defense against invasion by pathogens and for wound healing and repair. alphaDß$_2$ (CD11d/CD18) is the most recently discovered integrin, appearing restricted to subsets of macrophages and shown to be regulated during macrophage differentiation *In vitro*. In this study, we investigated the potential contribution of alphaDß$_2$ to the early phase of experimental silicosis in mice. **Methods:** alphaDß$_2$ knockout and wild type littermate (C57/Black 6) mice were instilled intranasally with crystalline silica particles (10 mg/50 µL). The analyses were performed on day 7 and included lung function (resistance and elastance) by invasive plethysmography (Fine-point Buxco System) and ii) leukocyte infiltration in the bronchoalveolar lavage (BAL). Total and differential leukocyte counts were performed in Neubauer chamber and cytocentrifuged smears coloured with May-Grunwald-Giemsa dye. Classical histological techniques were used and included morphology and morphometry (H&E and Picrus sirius) and immunohistochemistry. Collagen was quantified by Sircol method. All experimental procedures were performed in accordance with guidelines of the Committee on Use of Laboratory Animals of the FIOCRUZ (license L034/09). **Results:** Intranasal silica into the wild type mice led to an increase in the leukocyte numbers in the BALF, mainly macrophages and neutrophils, phenomenon which paralleled with a marked leukocyte infiltration and numerous granulomas presented in the lung tissue. The alphaDß$_2$ knockout mice exhibited a less intense macrophage infiltration (51%) in the BALF as well as reduced infiltration in the lung tissue. Attenuation of granuloma formation and tissue collagen deposition was also detected in the knockout mice stimulated with silica. As attested by immunohistochemistry, expression of TGFß in the lung tissue of the knockout mice was clearly reduced as compared to that of wild type. Interestingly, silica-stimulated mice showed an increase in the basal levels of lung resistance and elastance, response clearly decreased in the knockout animals. **Conclusion:** Our findings indicate that alphaDß$_2$ integrin seems to play a role in the early phase of lung inflammation caused by silica in mice and also offer evidence that macrophages are important and effective part of the process of immediate immunity responses such as silicosis. **Financial support:** CNPq, PAPES/FIOCRUZ, FAPERJ (Brazil).

04.056 Effect of ezetimibe on PLA2 inflammatory and catalytic activity. Marangoni FA, Antunes E, De Nucci G, Landucci ECT Unicamp - Farmacologia

Introduction: Secretory phospholipase A_2 ($sPLA_2$) is an enzyme that plays an important role in the pathogenesis of atherosclerosis and of adverse cardiovascular events. Ezetimibe is an inhibitor of cholesterol absorption in the intestine that is frequently added to statins in patients with lower than optimal levels of low-density lipoprotein (LDL) cholesterol. Although ezetimibe is very effective in providing additional lowering of LDL cholesterol when co-administered with statins, the effects of ezetimibe/statin co-administration beyond LDL lowering such as the effect on $sPLA_2$ remain unknown. Clinical studies, suggested that the combination of ezetimibe/atorvastatin, results in a synergic effect that leads to a decrease in s PLA_2 activity. We studied the role of ezetimibe in inhibit the enzymatic activity of PLA_2*In vitro* and *in vivo* using models of inflammation induced by PLA_2 from *Bothrops leucrurus* snake venom. **Methods:** Treated animals received ezetimibe orally (40 mg/kg /day) for 7 days before the experiment. Saline (0.9%; negative control animals) or PLA_2 from *Bothrops leucrurus* snake venom (300 mg/kg) were injected into the pancreatobilliary duct of anaesthetized rats in a steady manual pressure over a period of 60 sec. Four hour thereafter, the animals were sacrificed, and blood samples and pancreatic tissue were collected. The pancreatic plasma extravasation and pancreatic and pulmonary mieloperoxidase (MPO) activity were investigated. The effect of ezetimibe on the phospholipase catalytic activity *In vitro*, was performed according to the method described by Holzer and Mackessy (1996), modified for 96-well plate (Soto-Ponce et al., 2002). The substrate was 4-nitro-3-(octanoiloxi) benzoic acid. The PLA_2 from *Bothrops leucrurus* snake venom and Crotapotin (from *Crotalus terrificus cascavella*) were used with a concentration of 1 mg/ml and the ezetimibe with variable concentration of 1mM at 1nM. The samples were incubated with reaction buffer (0.1M Tris-HCl Ca^{2+} 0.01M pH 8) for 30 minutes. After incubation, the absorbance was determined at 425 nm. **Results:** Pretreatment of animals with ezetimibe did not affect the increased plasma extravasation or MPO levels in the pancreas and lungs. Ezetimibe also failed to affect the catalytic activity of PLA_2 from *Bothrops leucrurus* assayed *In vitro*. On the other hand, crotapotin (a PLA_2 inhibitor), used as a positive control, was inhibited by 52% the catalytic activity of the same PLA_2. **Conclusion:** Our studies indicate that ezetimibe neither inhibits the $sPLA_2$ activity nor presents anti-inflammatory activity in the model of pancreatitis used. **Financial support** : CAPES. Animal Ethics Committee: nº1460-1

04.057 Vanillic acid, an inhibitor of 5´-ectonucleotidase, attenuates plasma extravasation caused by *Bothrops alternatus* (Urutu) snake venom in rat skin. Marcelino-Pereira E, Silva IRF, Hyslop S Unicamp – Farmacologia

Introduction: Purines and related compounds can enhance vascular permeability and may be involved in local responses to venoms. We have previously observed that purines are involved in the enhanced vascular permeability caused by *Bothrops* venoms. Here, we show that vanillic acid, an inhibitor of 5´-ectonucleotidase, attenuates the increase in vascular permeability caused by *B. alternates* venom. **Methods:** Male Wistar rats (200-300 g) were anesthetized with sodium thiopental (50 mg/kg , i.p.) and plasma protein extravasation was measured in the shaved dorsal skin in response to *B. alternatus* venom (10 mg/site) and other agents injected intradermally (in 100 µl of Tyrode solution/site). Plasma protein extravasation was measured by the accumulation of human ^{125}I-serum albumin (2.5 mCi/rat) injected i.v. along with Evan's blue dye (25 mg/kg). Thirty minutes after test agent injection, the rats were killed with an overdose of anesthetic and the injected sites were punched out and counted for radioactivity, along with plasma samples. Plasma extravasation was expressed as the volume (µl) of plasma accumulated at each skin site compared to total counts in 1 ml of plasma. The results were expressed as the mean±SEM. Statistical comparisons were done using Student's unpaired *t*-test or ANOVA followed by Bonferroni's modified *t*-test, with $p<0.05$ indicating significance. The experiments were approved by an institutional Committee for Ethics in Animal Experimentation (CEEA/UNICAMP, protocol no. 1318-1). **Results:** *Bothrops alternatus* venom (10 mg/site, n = 5) caused an increase in vascular permeability (from 4.0±1.1 ml (basal) to 135.9±8.0 ml) that was not significantly altered by dialysis (138.4±12.3 ml) or ultrafiltration (Millipore filters) (125.2±8.9 ml) (n = 5 each); the filtrate from ultrafiltration did not increase permeability (10.8±2.9 ml compared with basal value of 6.6±2.5 ml). Vanillic acid (VA; 1 nmol/site) significantly attenuated the plasma extravasation caused by venom (10 mg/site; from 135.9±8.0 ml to 72.1±9.6 ml; $p<0.001$). Adenosine (100 nmol/site) caused plasma extravasation of 119.9±13.2 ml that was significantly attenuated (to 27.1±1.9 ml; n = 5) by VA. In contrast, VA had no effect on edema caused by inosine (100 nmol/site), a metabolite of adenosine (80.0±8.0 vs 79.5±5.8 ml in the absence and presence of VA; n = 5). VA also did not significantly attenuate the edema caused by monophosphate nucleotides (100 nmol/site, n = 5 each; AMP 60.5±4.5 vs 69.4±3.9 ml, CMP 85.8±10.4 vs 59.5±6.9 ml, GMP 79.0±9.7 vs 58.1±6.3 ml and IMP 68.2±7.5 vs 75.5±10.6 ml in the absence and presence of VA, respectively), bradykinin (30 nmol/site; 66.2±4.4 vs 96.2±18.1 ml in the absence and presence of VA; n = 5) or histamine (30 nmol/site; 64.0±4.5 vs 92.0±9.8 ml in the absence and presence of VA; n = 5). **Conclusion:** VA inhibited venom-induced edema, indicating a role for venom 5´-nucleotidase and/or endogenous 5´-ectonucleotidase in this response. The effect of VA was specific for the adenosine pathway since responses to other agonists were unaffected. The venom-induced edema did not involve low-molecular mass components of the venom. **Financial support:** CAPES

04.058 Meso-tetraarilporphyrins: **photodynamic effect on human keratinocyte cell viability**. Carrenho LZB[1], Slomp A[1], Ló SMS[1], Ducatti DRB[2], Duarte MER[2], Noseda MD[2], Gonçalves AG[1], Cabrini DA[3], Barreira SMW[1], Otuki MF[4] [1]UFPR – Ciências Farmacêuticas, [2]UFPR – Bioquímica, [3]UFPR – Farmacologia, [4]UEPG – Ciências Farmacêuticas

Introduction: Photodynamic therapy (PDT) is a treatment modality that uses light in appropriated wavelength, oxygen and a photosensitizer in order to produce reactive oxygen species, inducing cell death. It is indicated for treatment of skin hyperproliferative diseases such as skin cancer and psoriasis, since this treatment is capable to reduce cell viability inhibiting epithelial cells proliferation. In this study we evaluated the possible photosensitizer property of three meso-tetraarilporphyrins: 5,10,15-triphenyl-20(N-methylpyridinyum-4-il)porphyrin iodide (1), 5,10,15,20-tetrakis(N-methylpyridinyum-4-yl)porphyrintetraiodide(2), meso-tetrakis [2,3,5,6-tetrafluoro-4-(N-methylpyridinyum-4 ylsulfanyl)phenyl] porphyrin tetraiodide (3). **Methods:** For treatment with light or in the dark, concentrations of 0.1, 0.3, 1, 3 and 10 μM of the porphyrin derivatives were tested in the cell line of immortalized human keratinocytes (HaCaT) seeded in a concentration of $7x10^3$/ well in 96 well plates. To evaluate the cytotoxicity of the meso-tetraarilporphyrins, cells were initially exposed to treatment with the porphyrin derivatives for 24 hours in the dark. To irradiation studies, after treatment, cells were exposed to a white light from a compatible fiber optic probe (400-800 nm) attached to a 250 W quartz/halogen lamp (LumaCare®, USA, model LC122) at a fluence rate of 100 mW cm^{-2}. Porphyrins cytotoxicity with or without light incidence was accessed by the measure of cell viability through of MTT assay. **Results:** All tested porphyrin derivatives were able to reduce cell viability. Porphyrin 1 decreased cell viability with maximum effect of 75.3 ± 2.2% (0.3 μM; 30 min). Porphyrins 2 and 3 exhibited a pattern of activity concentration and time dependent, the higher the compound concentration and time of the light incidence, the greater the cytotoxicity induced by the compound. Porphyrin 2 decreased cell viability in 79.1 ± 0.4% at a concentration of 10μM after 30 minutes of irradiation; under the same conditions, porphyrin 3 was responsible for reduce 69.8 ± 1% of cell viability. **Discussion**: Porphyrins 1-3 were evaluated based on varying of concentration and irradiation time. The resulting data indicated that although in most cases the cytotoxicity was dependent on concentration, high concentrations can induce cell death, even in the absence of light, by other mechanisms than photodynamic properties. Time of irradiation seems to be responsible for cytotoxicity since it stimulates the production of reactive oxygen species and trigger mechanisms of cell death. Keratinocytes are the main constituents of skin epidermis and play an important role in chronic hyperproliferative skin diseases. The porphyrins presented in this work can be used as lead compounds to conduct further studies involving the synthesis of new porphyrins with greater specificity and selectivity in photodynamic therapy against various skin diseases. **Support**: CAPES, CNPq, Fundação Araucária and REUNI-UFPR.

04.059 Endogenous hydrogen sulfide modulates inflammatory cell infiltration and airway remodeling in the lung of allergic mice. Guedes CEV[1], Pereira JA[2], Mendes JA[1], Rocha T[2], Ferreira HHA[1] [1]USF – Inflammation Research, [2]USF – Multidisciplinary Laboratory

Background: Hydrogen sulfide (H_2S) is produced endogenously by three enzymes: CBS (cystathionine β-synthase), CSE (cystathionine γ-lyase) and 3-MST (3-mercaptopyruvate sulfurtransferase) and plays an important role in inflammatory respiratory diseases. The enzymes locations in the mammalian tissues are variable depending on the species and cell types, but the CSE expression in the lungs is predominant (Chen and Wang, *Respir Physiol Neurobiol* 2012, Mar 20 [Epub ahead of print]). Our previous studies showed that allergic mice treatment with exogenous H_2S donor caused a significant decrease in inflammatory cells as well as goblet cells proliferation, mucus formation and mast cells degranulation in lung tissue. It was also observed an inhibition on eosinophils and neutrophils infiltration in the bronchoalveolar lavage (BAL; Guedes et al.; Benetti et al., XXVI Annual Meeting Fesbe, 2011). **Objective:** This study was carried out to verify if the endogenous production of H_2S may similarly modulate the allergic pulmonary inflammation. The CSE expression was also analyzed in the mice lung tissue. **Methodology:** All animal care and experimental procedures were approved by Animal Ethics Committee of San Francisco University (license number 0021108). BALB/C mice were sensitized with s.c injection of ovalbumin (OVA) on days 0 and 7 and on days 14 and 15 were intranasally challenged with OVA, 2x/day. Half of OVA-sensitized mice received intraperitoneal treatment with the irreversible inhibitor of CSE enzyme, propargylglicine – PAG or with H_2S donor, NaHS, 30 minutes before each challenge. Forty eight hours after the first challenge, the animals were sacrificed; the BAL and lung were collected. The total and differential leukocyte counts were made in the LBA. The left upper lobe of each animal was removed and fixed in formalin for histological analysis and CSE immunohistochemical staining. The lung tissue sections were stained with: hematoxylin-eosin to evaluate the inflammatory cells infiltration; periodic acid-Schiff or toluidine blue to estimate the percentage of goblet cells as well as mucus and mast cell degranulation in the airways, respectively. The CSE immunohistochemistry was performed using the MaxHomo™ kit (Max Vision Bioscience). **Results:** Treatment of allergic mice with PAG was able to reverse inhibition of eosinophil and neutrophil migration to the lungs caused by the NaHS. In addition, a 100% increase in neutrophil content in the BALF was also observed in these animals, compared to the control group. No significant difference between control and PAG-treated mice was observed in relation to the airway remodeling index of peribronchiolar or perivascular inflammatory cells, mast cell degranulation or the number of goblet cells and mucus. All these indices were reduced by NaHS treatment. Immunohistochemical staining showed that the expressions of CSE were mainly located in airway endothelial cells, epithelial cells, smooth muscle cells and mononuclear cells infiltrate. **Conclusion:** Our results suggest that the H_2S endogenously produced by CSE enzyme has an anti-inflammatory role on inflammatory cell infiltration and airway remodeling in the lung of allergic mice. **Financial support:** FAPESP, CAPES and Biogen.

04.060 Sublingual ketorolac and sublingual piroxicam are equally effective for postoperative pain, **trismus**, **and swelling management in lower third molar removal**. Senes AM[1], Gonçalves PZ[1], Melo AO[1], Santos CF[1] FOB-USP – Biological Sciences

Objective: Lower third molar removal provides a clinical model for studying analgesic drugs. The present study's aim was to compare the clinical efficacy of sublingual ketorolac and sublingual piroxicam in managing pain, trismus and swelling after lower third molar extraction in adult volunteers. **Study design:** In this double-blinded, randomized, crossover investigation, 47 volunteers received for 4 days ketorolac sublingually (10 mg 4 times daily) and piroxicam sublingually (20 mg once daily) during 2 separate appointments after lower third molar extraction of symmetrically positioned lower third molars. The Institutional Ethics Committee approved the protocol of this study. All patients provided written informed consent during the pretreatment screening period before any study procedures were performed. A surgeon evaluated objective parameters (surgery duration, mouth opening, rescue analgesic medication, and facial swelling) and volunteers documented subjective parameters (postoperative pain and global evaluation), comparing postoperative results for a total of 7 days after surgery. The means of the objective and subjective parameters were compared for statistical significance ($P < .05$). **Results:** Volunteers reported low pain scores during the postoperative period when treated with either sublingual ketorolac or piroxicam. Also, volunteers ingested similar amounts of analgesic rescue medication (paracetamol) when they received either drug sublingually ($P > .05$). Additionally, values for mouth openings measured just before surgery and immediately after suture removal 7 days later were similar among volunteers ($P > .05$), and the type of nonsteroidal antiinflammatory drug (NSAID) used in this study showed no significant differences between swellings on the second or seventh days after surgery ($P > .05$). **Conclusions:** Pain, trismus, and swelling after lower third molar extraction, independent of surgical difficulty, were successfully controlled by sublingual ketorolac (10 mg 4 times daily) or sublingual piroxicam (20 mg once daily), and no significant differences were observed between the NSAIDs evaluated. **Acknowledgments:** FAPESP (2010/16985-8, 2011/00817-1 and 2012/02834-3)

04.061 ***Marcgraviaceae*-originated compounds reduce DENV-2 *In vitro* infection and MIF production in a human hepatocyte cell line (HUH-7)**. Fialho LG[1], Lima Júnior RS[1], da Silva VP[2], Torrentes-Carvalho A[1], Mello C[1], Corrêa G[1], Figueiredo MR[2], Kubelka CF[1] [1]IOC-Fiocruz, [2]ITF-Fiocruz

Introduction: Dengue fever is still considered a neglected disease by the pharmaceutical industry, since there are no sufficient efforts in the drug development. The identification of compounds with immunomodulatory properties is extremely important for the treatment of dengue, since the disease severity is directly related to exacerbated production of an immunological response, mainly cytokines that may lead to hemodynamic and coagulatory disorders. The macrophage migration inhibitory factor (MIF) has been described as an aggravating factor, increasing the permeability of endothelial cells (Chuang *et al*. Cytokine (54): 222, 2011). *Marcgraviaceae,* a family of the Brazilian flora, has been evaluated by its biodynamic activities which presented an anti-inflammatory effect (Rocha, M; Dissertação, Instituto Oswaldo Cruz; 2002). **Methods:** In order to evaluate the immunomodulatory and antiviral activities of plant compounds, it was used an *In vitro* model of infection with a hepatocyte cell line (Huh-7). These cells were infected with DENV-2 (strain 16681) and incubated for 48 hours with a buthanolic fraction, and four of its subfractions, derived all from a crude ethanolic leave extract of a *Marcgraviaceae* species. The antiviral activity was determined by viral antigen (Ag DENV) detection in Huh-7 cells, using flow cytometry. The supernatants of the Huh-7 cultures were assayed to evaluate the immunomodulatory activity of the fractions/subfractions by the determination of MIF, using ELISA. **Results:** We observed that the buthanolic fraction and the 89-98 subfraction reduced viral infection and MIF production showing, therefore, both antiviral and immunomodulatory activities. In addition, the 26-30 and 40-43 subfractions exhibited only antiviral effect detected by the reduction of infection rate and the 99-134 subfraction exhibited only an immunomodulatory effect, reducing MIF production. **Discussion:** These results indicate that the buthanolic fraction and 89-98 subfraction may be potential candidates for the production of herbal medicines for dengue treatment, since they reduce both the virus infection and MIF levels - one of the most important cytokines involved in the disease severity. **Financial support:** FIOCRUZ; CAPES; CNPq; FAPERJ.

04.062 Zymosan injected into air pouches of rats induces fever dependent on prostaglandins but not on neural pathways. Marquiafável FS[1], Malvar DC[2], de Melo MCC[1], Souza GEP[1] [1]FCFRP-USP – Física e Química, [2]FMRP-USP – Farmacologia

Introduction: Fever is defined as controlled elevation body temperature in response to inflammation or invasion by infectious agents stimulating host defense via interaction with associated membrane pattern-recognition receptors promoting the release of endogenous pyrogens, such as cytokines, chemokines and peptides. These mediators, in turn, are responsible for the trigger of PGE_2-dependent or -independent fever. It has been proposed that the pyrogenic signaling may be transmitted from periphery to the brain by humoral and/or neural pathways. Previously, we observed that prostaglandins are involved in the fever induced by intra-articular injection of zymosan in rats while sciatic nerve does not (Kanashiro, Am J Physiol Regul Integr Comp Physiol 296:1631, 2009). So, the aim of this study was to investigate the involvement of prostaglandins and the contribution of neural pathways in the fever induced by zymosan injected into the air pouches in rats. **Methods:** 1th day: under deep anesthesia (xylazine 20mg.kg^{-1}+ ketamine 58mg.kg^{-1}, ip) 20ml of sterile air was injected subcutaneously in the back of male Wistar rats (180g) and the transmitter was set in ip cavity. 3th day: 10ml of sterile air was injected into the air pouches (ap.). 6th day: the animals received vehicle (saline), celecoxib (1, 2.5, 5mg.kg $^{-1}$, po), dipyrone (60, 90, 120mg.kg^{-1}, i.p) or ropivacaine (2.5, 5, 10mg.kg^{-1}, injected into or around ap.) 30 min before ap. injection of zymosan (0.5, 1, 4 mg.ml^{-1}, 1ml) or saline (control group). The body temperature (^{o}C) was measured every 15 min up to 6h by radiotelemetry (Ethical Commission Protocol n^{o} 12.1.322.53.2 – CEUA/RP-USP). **Results:** Zymosan promoted a dose-dependent febrile response that began at 2.5h, peaked at 3.5h and remained for 6 hours. Regardless the doses, celecoxib inhibited while dipyrone reduced dose-dependently zymosan-induced fever (1mg.ml^{-1}). Ropivacaine itself did not change the body temperature in the absence of the stimulus. However, administrated into or around the ap. 30 min prior zymosan, it dose-dependently increased the febrile response.

Effect of dipyrone, celecoxib and ropivacaine on fever induced by zymosan injected into air pouch (ap.) in rats.

Treatment** (mg.kg^{-1})		zymosan (mg.ml^{-1}, ap.)	Δ °C* (3.5h)
saline		4	1.79±0.09
		1	1.34±0.14
		0.5	1.24±0.13
		saline	0.06±0.04
Celecoxib (po)	1	1	0.09±0.08
	2.5		0.26±0.03
	5		0.16±0.13
	saline		1.18±0.10
		saline	0.03±0.05
Dipyrone (ip)	60	1	0.75±0.13
	90		0.25±0.06
	120		0.20±0.06
	saline		1.64±0.12
		saline	-0.09±0.13
Ropivacaine (ap.)	2.5	saline	-0.22±0.07
	5		-0.05±0.06
	10		-0.08±0.05
	saline		-0.05±0.05
		1	-0.20±0.07
	2.5		1.30±0.10

	5		1.10±0.14
	10		1.52±0.15
Ropivacaine (around ap.)	2.5	1	1.35±0.06
	5		1.21±0.05
	10		1.11±0.11
	saline		1.26±0.13
		saline	-0.05±0.05
	5		-0.32±0.18
	10		-0.27±0.08

*****mean ±SEM; ******animals *per group:* 7-10

Discussion

As observed in knee joints, zymosan injected into the air pouch of rats induces a dose-dependent fever which was reduced by celecoxib and dipyrone suggesting the involvement of prostaglandins. Furthermore, if involved, neural pathway works as modulator instead of promoter of zymosan-induced fever once ropivacaine enhanced this response. Further studies are necessary to better elucidate the mediators and mechanisms involved in this response. **Financial support**: CNPq.

04.063 Down-modulation of activated human neutrophil by LMW-Fucoidan. Frony AC[1], Moraes JA[1], Boisson-Vidal C[2], Barja-Fidalgo TC[1] [1]UERJ – Biologia Celular, [2]INSERM

Introduction: During migration, neutrophils (PMN) interact with several mediators, which lead to their activation, interfering with cell survival and the inflammation resolution. Fucoidans are sulfated polysaccharide found in brown algae, able to inhibit selectin-mediated events, such as the leukocyte rolling. A low-molecular-weight fucoidan fraction extracted from the brown algae *Ascophyllum nodosum* (LMW-Fuc) exhibit potent antithrombotic, but low anticoagulant activities and potent proangiogenic properties, although its effects on inflammatory cells is still unknown. We aimed to evaluate the effect of LMW-Fuc on activated PMN. **Methods:** Chemotaxis (1h) of isolated human PMN (Percoll gradient) was performed in Boyden chamber. Apoptosis (20h) was assessed by Annexin V (FACS), JC1 (Envision) and morphological analysis (microscopy). Actin cytoskeleton rearrangement was analyzed by Falloidin-rhodamin staining (Fluo microscopy). ROS production was quantified by lucigenin, luminol and CM-DCFDA (Envision), according the ROS localization to be analyzed. AKT/Bad expression was evaluated by immunoblotting (Western Blotting). Calcium influx was analyzed by Fura-2AM (Envision). AnnexinV^+-microparticles was quantified in FACS. **Results:** LMW-Fuc (10 µg/mL) inhibited PMN migration induced by LPS, fMLP or of PMN primed with LPS and further challenged to fMLP. Corroborating this data, in PMN activated with LPS/fMLP the LMW-Fuc attenuated the induced alterations on actin cytoskeleton dynamics and inhibited AKT phosphorylation. We also observed that LMW-Fuc was able to accelerate apoptosis of PMN treated with LPS, fMLP or primed with LPS and further treated with fMLP and, corroborating this result, LMW-Fuc prevented Bad degradation induced by LPS/fMLP treatment. Then we showed that LMW-Fuc was able to inhibit extracellular, but not the intracellular ROS production and decreased intracellular Calcium release induced by LPS/fMLP treatment. However, LMW-Fuc was not able to internalize or desensitize FPR1 (fMLP receptor). Furthermore, LMW-Fuc inhibited the release of microparticles by PMN stimulated with LPS/fMLP. **Conclusion:** Together the data indicate that LMW-Fuc presents potent anti-inflammatory properties that might be potentiated by its ability in inhibit microparticles release. **Funding Support**: FAPERJ, CAPES, CNPq.

04.064 IL-22 modulates IL-17A production and controls inflammation and tissue damage in experimental dengue infection. Marques RE[1], Guabiraba R[1], Besnard AG[2], Conceição TM[3], Da Poian AT[3], Souza DG[4], Ryffel B[2], Teixeira MM[1] [1]ICB-UFMG – Bioquímica e Imunologia, [2]Université d'Orléans – Molecular and Experimental Immunology and Neurogenetics, [3]IBqM-UFRJ, [4]ICB-UFMG – Microbiologia

Introduction: Dengue virus (DENV), a mosquito-borne flavivirus, is a public health problem in many tropical countries. IL-22 and IL-17A belong to a new class of cytokines and are key molecules in several infectious and inflammatory diseases, and their role in dengue pathogenesis is not understood. **Methods:** We have assessed the contribution of IL-22 and IL-17A in the pathogenesis of experimental dengue infection using a mouse-adapted DENV serotype 2 strain (P23085) that causes a disease that resembles severe dengue in humans. Upon infection, parameters of disease such as mice survival, cytokine production, viral load in tissues and activated leukocyte populations were evaluated. Experiments *In vitro* were also performed to evaluate IL-22 direct effects on the HepG2 cell line. All experimental procedures were approved and complied with the French government's ethical and animal experiment regulations (CLE CCO 2009-013). **Results:** We showed that IL-22 and IL-17A are produced upon DENV-2 infection in mice. Infected IL-22$^{-/-}$ mice had increased lethality, neutrophil accumulation and pro-inflammatory cytokines in tissues, notably IL-17A, when compared to infected C57BL/6 wild-type (WT) mice. Viral load was slightly increased in spleen and liver of infected IL-22$^{-/-}$ mice. There was also more severe liver injury, as seen by increased AST/ALT levels and tissue histopathology. Activation of CD4^{+}, CD8^{+} and NKT cells was greater in IL-22$^{-/-}$ mice than in WT mice. In parallel, we showed that DENV infected HepG2 cells treated with rhIL-22 had reduced cell death and decreased IL-6 production. IL-17RA$^{-/-}$ mice were slightly protected upon DENV infection and IL-17-neutralizing-antibody-treatment partially reversed the phenotype observed in IL-22$^{-/-}$ infected mice. **Discussion:** These new data suggests that IL-22 partially controls the excessive inflammatory response to dengue infection by modulating IL-17A production. Strategies interfering with the balance between IL-22 and IL-17A levels may represent an important strategy to reduce inflammation and tissue injury associated to severe dengue infection, and probably to other flavivirus infections. **Financial Agencies**: CNPq, FAPEMIG, INCT em Dengue (Brazil). ANR and CNRS (France).

04.065 Molecular features of anemia and type 2 diabetes. Faria TF[1], Silva SV[2], Barja-Fidalgo TC[2], Citelli M[3] [1]UERJ – Nutrição, [2]UERJ – Biologia Celular, [3]UERJ – Nutrição Básica e Experimental

Iron deficiency and type 2 diabetes caused by obesity are public health problems. Although there are some clues that the inflammatory state caused by obesity affects iron metabolism, little is known about the molecular mechanisms that surround them and about the additional effects caused by diabetes. Our aim was to describe the molecular effects of obesity and type 2 diabetes on iron metabolism. During 32 weeks, C57Bl6 male mice received a control diet (CONT) or a high fat diet (HFD). In a third group (HFD/HFD), the mother received a HFD throughout the life, including its pregnancy and lactation and the offspring was fed a HFD for 32 weeks. After this period, the weight of animals from HFD groups was increased (CONT = 29.5 g + 2.2; HFD = 33.0 g + 1.9; HFD/HFD = 41.0 g + 4.6) and the GTT (glucose tolerance test) revealed they had insulin resistance. Small intestine and liver were used in the gene expression analysis by real time PCR. ELISA assay showed that both groups on HFD had approximately 3 times higher levels of leptin, a proinflammatory cytokine capable of activating hepcidin transcription. Hepcidin is a hormone that regulates iron homeostasis and acts by preventing the iron to be transported from enterocytes to the portal circulation and from macrophages to the bloodstream. Its liver expression was about 26% greater in the HFD group. Accordingly, liver ferritin gene expression was also significantly increased. However, the expression of DMT1 (divalent metal transporter), a transmembrane permease that transports iron through the intestinal surface, did not differ between groups. Together, the data corroborate that obesity and type 2 diabetes may affect iron metabolism. Ethics committee: CEA/0452009 **Financial support**: FAPERJ

04.066 Evaluation of anti-inflammatory activity of α-phellandrene *in vivo* and *ex vivo* models. Siqueira HSS[1], Sousa-Neto BP[1], Sousa GA[1], Rocha FTA[1], Amorim LV[1], Rodrigues KAF[1], Oliveira FA[1], Oliveira RCM[1], Sousa DP[2] [1]NPPM-UFPI, [2]UFS – Química

Introduction: α-phellandrene (α-FEL) a cyclic monoterpene is present in the chemical composition of essential oils from several species that present anti-inflammatory activity such as *Zingiber officinale* Roscoe. In previous study we demonstrated the antiedematogenic effect of α-FEL in various models of edema. This study aims to evaluate the anti-inflammatory potential of α-FEL in models of inflammation of air pouch induced by carrageenan and mast cell degranulation induced by compound 48/80 (C48/80) in rats. **Methods**: Male Wistar rats (150-210 g, n = 8/group, approved by Ethics committee of Animal Experimentation-CEEA/PI n°. 010/11) were used. The animals were anesthetized (ketamine and xylazine 50 and 5 mg/kg , i.m., respectively) and were injected with 20 mL of sterile air administered in the intra-scapular area of the back and maintained by re-inflation with 10 mL of air 3 and 6 days later. On the sixth day the animals were treated p.o. with vehicle (3% Tween 80), α-FEL (50, 100 and 200 mg/kg) or dexamethasone (0.5 mg/kg). After one hour was administered 0.1 mL of carrageenan (2%) into the pouche and four hours later the animals were euthanized (sodium pentobarbital 100 mg/kg , i.p.); the contents of the air pouche were removed using Pasteur pipette after injection of 10 mL of PBS. The exudate cells were separated and the total number of leukocytes was counted (mm^3). In the model degranulation of mast cells *ex vivo*, rats were divided into groups and treated for p.o. with saline 0.9%, vehicle (3% Tween 80), α-FEL (50, 100 and 200 mg/kg) or ketotifen (2 mg/kg). After one hour, the animals were euthanized, the mesentery removed and placed in test tubes containing Ringer's solution (10 mL). In groups treated with vehicle, α-FEL or ketotifen the degranulation of mast cells was induced by incubation of the mesentery with 100 µL C48/80 (0,4 µg/mL). To the group saline was added 100 µL of distilled water. After 30 minutes of incubation, the mesenteries were mounted on glass slides and stained with toluidine blue (0.1%). The counting of intact and degranulated mast cells was performed in five of each slide and the result expressed as the % of degranulated mast cells. Data were expressed as mean±standard error of mean. Statistical analyzes were performed using ANOVA (one way) followed by Student Newman Keul test. **Results and Discussion**: The results demonstrated that α-FEL (50, 100 and 200 mg/kg) inhibited ($p<0.001$) the migration of leukocytes in the exudate (440.0±57.9, 980.0±153.8 and 560.7±57.9, respectively) when compared to saline group (2237.5±42.7). Similarly the group treated with dexamethasone (180.0±33.9, $p<0,001$). In *ex vivo* model, α-FEL (50, 100 and 200 mg/kg) and ketotifen (2 mg/kg) reduced the degranulation in 63.5±2.1; 27.4±1.8; 21.6±1.7 and 19.5±1.9 ($p<0,001$), respectively, when compared to vehicle group (85.4±1.8). These results indicate that α-FEL interfere with the inflammatory mediators involved in the pathogenesis of peritonitis induced by carrageenan and in the stabilization of mesenteric mast cells suggesting that these actions may be responsible for the anti-inflammatory effect observed with this monoterpene. **Financial Support**: UFPI / CAPES

04.067 N-acetylcysteine prevents and reverses the inhibitory effect of *in vivo* lipopolysaccharide on platelet aggregation. Anjos DJ, Silverio-Mendes CB, Bonfitto PHL, Antunes E, Marcondes S Unicamp – Farmacologia

Introduction: Lipopolysaccharide (LPS) interacts with a number of cell types such as endothelial cells, macrophages, leukocytes and platelets, causing the release of different substances including cytokines, chemokines, reactive oxygen (ROS) and nitrogen species (RNS). These reactive species are important to modulate the cell function, and in combination with endogenous antioxidant systems are crucial to the maintenance of the redox balance in the organism. N-acetylcysteine **(**NAC) is an antioxidant widely used in studies of oxidative stress and it is able to inhibit different LPS responses by reducing ROS generation and/or by increasing glutathione production. Therefore, the objective of the present work was to investigate the effect of NAC treatment in platelets of rats before or after LPS exposition. **Methods:** The present study was approved by the Committee for Ethics in Animal Research (State University of Campinas – UNICAMP, protocol number 2338-1). Male Wistar rats (250-320 g) were injected i.p. with saline, NAC (150 mg/kg) or LPS (from *E. coli*, 1 mg/kg) as control groups and after 48h arterial blood was collected in ACD-C (9:1 v/v). In some experiments, rats received a single injection of NAC in different time-point after (30 min or 6h) or before (30 min or 6h) LPS treatment. Platelet aggregation was measured in a two channel aggregometer and the assays were carried using ADP (5 µM). Cyclic GMP levels were measured using an enzyme immunoassay kit. **Resultas:** *In vitro* addition of ADP produced significant washed platelet aggregation in saline-injected rats (50±5% of aggregation, n = 6). Similar results were obtained when the rats were injected with NAC. In vivo pre-treatment with LPS caused a significant reduction in ADP-induced platelet aggregation within 48 h post-LPS administration (36% of inhibition). NAC injection 30 min or 6h after LPS-treatment reversed the inhibitory effect of LPS on platelet aggregation. Similarly, the inhibitory effect of LPS on platelet aggregation was prevented when the rats were injected with NAC 30 min before LPS-treatment. However, ADP-induced platelet aggregation of rats injected with NAC 6h before LPS administration was not modify compared to the rats injected only with LPS. Platelet activation of LPS-treated rats at 48h caused a significant increase of cGMP production when compared to platelets of saline-injected rats (7.5 ± 2 pmol/ml and 3.9 ± 1 pmol/ml in LPS- and saline-injected rats, respectively; n = 4). Treatment of rats with NAC decreased 40% cGMP production compared to the saline-injected rats. The increased cGMP production in platelets of LPS-treated rats were reversed by NAC injected 30 min after LPS administration. **Conclusion:** Our findings showed that NAC prevents and reverses the effects of in vivo LPS on platelet activity. This improvement of platelet aggregation and cGMP production could be caused by direct action of NAC on platelets or by restoring the redox state of the animals. **Supported by:** CAPES

04.068 The activity of phenolic acid derivates from methanol extract of anacardiaceae family in acute airway allergic inflammation. Cavalher-Machado SC[1], Noenta-Lima NR[1], Rosas EC[1], Silva JD[2], Rocco PRM[2], Henriques MGMO[1]
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Introduction: It is well established the eosinophils influx at inflammatory sites during allergic disorders. *Schinus terebinthifolius* Raddi is a native plant from America widely used in folk medicine. We previously described the anti-allergic effect of acetate fraction obtained from their leaves in mouse pleurisy model inhibiting eosinophil influx. In this study we aimed to determinate the effect of phenolic acid derivates from Anacardiaceae fraction using a murine airway allergic inflammation model, observing the cellular influx to the mice lung, the lymphocytes includes in these mononuclear cells and the lung mechanics and morphometry. **Methods**: CL57BL-6 mice were sensitized on day 0 and 14 by subcutaneous injection of ovalbumin and $Al(OH)_3$, followed by three intranasal challenges (on pos-immunization days 21 to 23) with 4 mg/mL of ovalbumin delivered into the nostrils, under anaesthesia. The orally treatment (Methyl gallate 10 mg/kg) was administrated starting from the first day of challenge, until the third day, occurring 1 h before this. Dexamethasone (1 mg/kg) was used as a reference drug. The bronchoalveolar lavage fluid (BAL), the lymphocyte analyzed, by flow cytometry, and the lung mechanics and histology were performed 24 h after the last challenge. The levels of CCL5 in the cell-free BAL fluid were evaluated by ELISA. All experiments were performed in accordance with the Committee on Ethical Use of Laboratory Animals of Fiocruz (CEUA025/09). **Results:** Oral pre-treatment of sensitized CL57BL-6 mice with Methyl gallate (10 mg/kg) inhibited total leukocyte, mononuclear cells, neutrophil and eosinophil accumulation in bronchoalveolar lavage fluid (BAL) after OVA challenge (4 mg/mL, i.n.; from 5.96±1,03 to 0.91±0.05 $x10^5$/mL, from 2.59±0.52 to 0.79±0.08 $x10^5$/mL, from 0.87±0.24 to 0.04±0.05 $x10^5$/mL and 2.49±0.75 to 0.07±0.05 $x10^5$/mL, respectively). It was also observed that this treatment significantly decrease the levels of CCL5 in the BAL (from 0.015±0.005 to 0.002±0.006 ng/mL). Analyzing the BAL mononuclear cells population was observed that the treatment with Methyl gallate, inhibited the numbers of lymphocytes such as: $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells (60.7 and 75.4% respectively). It also decreased the expression of $CD3^+CD25$ and $CD3^+CD122^+$ (59.4 and 47.6% respectively) and the number of $CD3/\gamma\delta^+$ T cells (75.8%). Analyzing the lung mechanics and histology it was observed that Methyl gallate treated group decreased the bronchoconstriction index (34%), fraction area of alveolar collapse (67%), cellular infiltration in lung tissue (29.2%) and collagen fiber content in airway and lung parenchyma (17.2% and 14.9%; respectively). **Discussion:** We can suggest that the diminished eosinophils observed in BAL fluid, seems to be related to the lower number and activation of lymphocytes reaching the inflammation site, and directly related with the lower levels of CCL5 number quantified in BAL fluid suggesting an anti allergic and an immunoregulatory effect to phenolic acid derivates from Anacardiaceae fraction, observed after the mouse antigen challenge. **Sources of Research Support:** CNPq; FAPERJ

04.069 The anti-inflammatory effects of methyl ursolate derived from ursolic acid apple peel (*Malus domestica* Borkh.). Padua TA[1], Abreu BSSC de[1], Rosas EC[1], Siani AC[1], Nakamura MJ[1], Valente LMM[2] [1]ITF-FIOCRUZ, [2]UFRJ – Química

Introduction: Ursolic Acid (UA) a pentacyclic triterpene carboxylic acid is found in a large number of medicinal herbs, and other plants (1). Several biochemical and pharmacological effects of UA such as anti-inflammatory, antioxidant, anti-proliferative, among others properties are reported in a number of studies (2). The Fuji apple peels (*Malus domestica*, Rosaceae) is an abundant source of UA (3, 4), however, extracts from those peels have high viscosity and low solubility. To solve this problem the crude extracts can be chemically modified to generate UA derivatives more pure and soluble. The aim of this study is investigate the anti-inflammatory properties of Methyl Ursolate (MU) a derivative of the extract from Fuji apple peel. **Methods:** The Methyl Ursolate (MU) was obtained by the exhaustive methylation of the crude EtOH-AcOEt extract followed by column chromatography. The anti-inflammatory effects of UA and MU are evaluated by paw oedema, pleurisy and experimental arthritis models. Male Swiss mice were submitted to a dose dependent oral pre-treatment with MU (12,5 , 25 or 50 mg/kg) or AU (50 mg/kg) and paw oedema was induced by an intraplantar injection *(i.p.)* of zymosan (100µg/paw). The oedema was measure by the difference between stimulated and non-stimulated paw in the plethysmograph. The pleurisy and the experimental model of arthritis were performed in male C57/BL-6 mice which were pre-treated with AU and MU (50 mg/kg) and stimulated with zymosan by intrathoracic injection (i.t., 100µg/cavity) or intrarticular injection (i.a., 500µg/25µL). Diclofenac and Dexamethasone were used as inhibitor of reference. The pleurisy was assessed 4 hours after the stimulus. The thoracic cavities were washed with PBS/EDTA 10 mM and the pleural washes were used to analyze cell migration and protein extravasation. The synovial oedema was measured 6 hours after the stimulus and the intrarticular cavities were washed with PBS/EDTA 10 mM for cell migration analysis. Statistically significant differences between groups were determined using one way ANOVA with Newman-Keuls-Student post-test. **Results:** The intraplantar injection of zymosan induced an oedema (4h) that was reduced by MU and AU (both 50 mg/kg) in 44% and 46% respectively. After 4 hours zymosan-induced pleurisy there was an increase of leukocytes and protein extravasation. The pretreatment with AU and MU did not inhibit the leukocyte influx, and only the MU was able to inhibit the protein extravasation into the thoracic cavity. Both AU and MU inhibited in 47% and 41% the tibio-femoral junction oedema, in 52% and 74% the leukocyte influx and in 56% and 80% the neutrophil influx respectively into synovial cavity 6 hours after the zymosan stimulus. **Discussion:** Both AU and MU presented anti-inflammatory effects in distinct in vivo models. Only MU inhibits the protein extravasation in the pleurisy, showing also a more potent inhibition on cell migration in experimental arthritis. **References:** 1- Bringe et al. (2006) Phytochemistry 67, 161–170. 2- Rahul Checker et al. (2012) Plos one 7, 1-15 3- Frighetto N. et al. (2008) Food Chemistry 106, 767–771. 4- Jäger S. et al. (2009) Molecules 14, 2016-2031. **Support:** CNPq/Proc. 474751/2009-0. CEUA/FIOCRUZ: 052/08

04.070 Carbon nanotubes induce acute and chronic lung inflammation but little fibrogenic effects. Lima BHF[1], Lopes GAO[1], Russo RC[2], Teixeira MM[1] [1]UFMG – Bioquímica e Imunologia, [2]UFMG – Fisiologia e Biofísica

Introduction: Carbon nanotubes (CN) are carbon allotropes that belong to the fullerene family. Since their discovery in the 90's, applications for their usage in various fields including biology were proposed and their production notably increased (Carbon nanotubes: properties and applications, 2006) increasing its exposure to people that produces, manipulates or even live nearby carbon nanotubes factories. Due to their asbestos-like aspect, questions on the nanocompounds safety have been raised but little information about their toxicity is available. So, the aim of this work was to study the possible toxic effects of multiwall carbon nanotubes in mice lungs and to assess whether *In vitro* experiments can predict *in vivo* toxicity**. Methods:** For *in* vivo experiments, female C57BL/6 mice were intranasally instilled with 50 µg of CN in PBS. 1; 3 and 30 days after instillation the broncoalveolar lavage (BAL) and the lungs were collected for total and differential cell count, ELISA (IL-1β), western blotting, MPO, NAG and hidroxiproline assays and tissue histology. For *In vitro* experiments, cell lineages (RAW 264.7 and THP-1) were incubated and then stimulated with CN (1, 10 and 100 µg/mL in culture medium). 24 hours after stimulation the cell supernatant was collected to evaluate NO production and IL-1β levels. Intracellular ROS was measured by fluorescence in RAW 264.7 cells incubated with DCFDA, immediately before stimulation with CN 100 µg/mL. All the experimental procedures were approved and complied by the local ethical committee on animal experimentation (CETEA 191/10). **Results:** Our results show that CN exposure induces acute neutrophil and mononuclear cells transmigration to the airways by the 1st post instillation. Moreover, CN is able to induce chronic accumulation of neutrophils in lung parenchyma, by the 30th d.p.i. CNs were also able to induce IL-1β production in the BAL and lung parenchyma and promote pro-caspase 1 conversion to active caspase-1 by the 3rdd.p.i. After 30 days of exposure, CNs could induce little lung fibrosis, reaching only the perivascular and peribronchiolar regions. *In vitro*, our results also demonstrate that CNs induced nitric oxide production after 24 hours of stimulation even in very low concentrations. Furthermore, they were also able to induce a rapid and robust intracellular ROS production. CNs also induced the production of IL-1β by THP-1 cells after 24 hours of stimulation. **Discussion:** Our results demonstrate that this specific CN structure is able to interact with cells in the lungs of mice, causing acute and chronic inflammation, probably by the inflammasome pathway, but mild fibrosis. Moreover, *In vitro* experiments seem to be reliable to predict lung toxicity although current effort is to standardize these *In vitro* experiments. Altogether, these results indicate that CNs are a potential inhaled biohazard for those who are directly exposed to them as it may cause lung inflammation. This inflammation could also lead to worse results when associated with other factors such as metabolic disorders, pollutants (cigarette smoke) and/or pathogenic agents. **Financial support:** CNPq, NanoValid, CAPES, FAPEMIG,

04.071 Involvement of prostaglandins and substance P on zymosan induced febrile response. Bastos-Pereira AL, Fraga D, Zampronio AR UFPR – Farmacologia

Introduction: The most common model to induce fever in animals is the systemic administration of *E. coli*-derived LPS, a pathogen-associated molecule pattern (PAMP) which acts as a TLR4 agonist. LPS can induce fever by the activation of different pathways, for example those involving prostaglandins (PG) and substance P (SP). However, it is not entirely known if the same pathways induced by bacteria products are also involved in the development of fever induced by other agents. This study investigated the involvement of PGs and SP on the febrile response induced by another PAMP: Zymosan, a fungal cell wall preparation derived from *Saccharomyces cerevisiae* that acts as a TLR2/TLR6 agonist. The results were compared to those of LPS. **Methods:** 5 days prior the experiments, intraperitoneal (ip) dataloggers were implanted in male Wistar rats (180-220g) for temperature measurement. An intracerebroventricular (icv) cannula was implanted for drug administration. Body temperature was measured at 15 min intervals, 2h before the PAMP injection up to 6h. The non-selective ciclooxigenase inhibitor Indomethacin was administered (2 $mg.kg^{-1}$, ip) 30 minutes before the PAMP administration. Similarly, a selective NK1 receptor antagonist (SR140333) was administered icv (3µg) 30 minutes before the PAMP administration. Control groups received SR140333, indomethacin or the respective vehicle. After 30 min the animals received vehicle, Zymosan (3 $mg.kg^{-1}$, ip) or LPS (50 $\mu g.kg^{-1}$, ip). All experiments were conducted at 28±1°C. Only the data belonging to animals that presented basal temperatures between 36,8ºC and 37,4ºC was considered. Statistical analysis was performed using One-way ANOVA, followed by Bonferroni´s test. Data is presented as media ± standard error of the area under curve, in arbitrary units (AU), considering the change in body temperature 1 to 6 hours after the PAMP administration. All procedures were approved by the UFPR Ethical Committee in Animal Use, under protocol number 384. **Results:** Administration of LPS induced a significant increase in body temperature (5.8 ± 0.5 AU) as compared to the Control group (1.3 ± 0.9 AU). Administration of Indomethacin reduced this febrile response by 43,7% (3.2 ± 0.6 AU). Zymosan administration also augmented significantly the body temperature (2.8 ± 0.4 AU), and indomethacin administration reduced this febrile response by 52,2% (1.3 ± 0.2 AU). Concerning Substance P experiment, a febrile response was also observed with the administration of LPS (5.2 ± 0.3 AU) and Zymosan (4.2 ± 0.3 AU) as compared to the Control Group (1.4 ± 0.2 AU). Pretreatment of the animals with the NK1 receptor antagonist SR140333 reduced these febrile responses by 34,9% (3.4 ± 0.4 AU) and 30,1% (2.9 ± 0.3 AU) respectively. **Discussion:** These results suggest that PG and SP are not only involved in the febrile response induced by LPS, but they also seem to be important mediators involved in the development of fever induced by Zymosan. Indeed, the complete reversal of the febrile response induced by Zymosan upon indomethacin administration reveals the important role that prostaglandins seem to play in the febrile response induced by this PAMP. Currently, our research team is investigating other mediators, both peripheral and central, which may be involved on the febrile response by Zymosan. **Acknowledgements:** REUNI, CNPq and Araucária Foundation, for Financial support.

04.072 Effect of mangiferin on pulmonary function and remodeling in a murine model of asthma. Vieira AB, Athar CVA, Cotias AC, Pão CRR, Serra MF, Martins PMRS, Martins MA IOC-Fiocruz – Inflammation

Introduction: Allergic asthma is a disorder characterized by chronic lung inflammation, reversible airway obstruction and increase in airway hyper-responsiveness to nonspecific stimuli. Mangiferin, a xanthone isolated from *Mangifera Indica L.* and other plants, has a broad spectrum of effects, including anti-inflammatory and anti-allergic properties. Recently, its anti-inflammatory action was demonstrated in a murine model of asthma but parameters related with pulmonary function and remodeling remain unknown. The present study aimed to evaluate the effect of mangiferin on pulmonary function and remodeling parameters in a murine model of asthma. **Methods:** Separate groups of A/J mice were subcutaneously sensitized with 50 µg of ovoalbumin (OVA) adsorbed on aluminium hydroxide (5mg/ml) on day 0 and 14. Five days after the booster, mice were challenged with intranasal instillation of 25 µg of OVA dissolved in 25 µl of saline for two consecutive days. Mangiferin (50 mg/kg) was administered via oral route 1 h before provocations. Lung function, eosinophyl infiltration, eotaxin-1, eotaxin-2 and IL-4 generation and peribronchial fibrosis were evaluated 24 h after the last OVA challenge. All procedures involving care and use of laboratory animals were approved by the Animal Ethics Committee of the Fiocruz (n° LW23/10). **Results and Discussion:**OVA-exposed mice revealed lung eosinophilic inflammation and airway hyper-reactivity, accompanied by subepithelial collagen deposition. Treatment with mangiferin prevented allergen-induced changes in airway hyperresponsiveness and extracellular matrix deposition. Mangiferin reduced in 66 % eosinophil infiltration into peribronchiolar space and decreased the levels of eotaxina-1 (31,57%), eotaxina-2 (53,5%) and IL-4 (36,0%) in the lung tissue. Our results suggest that mangiferin can prevent crucial features of atopic asthma including inflammation, airway hyper-reactivity and tissue remodeling in the lung, and therefore should be further evaluated as a putative candidate in asthma therapy. **Financial support:** CAPES, FAPERJ e CNPq.

04.073 CCR5 expression on neutrophils plays a protective role during experimental sepsis. Castanheira FVS, Sônego F, Kanashiro A, Czaikoski PG, Cunha TM, Alves-Filho JC, Cunha FQ USP-FMRP – Farmacologia

Introduction and aim: Sepsis is a systemic inflammatory response resulted from the inability of the innate immune system to control infections being the survival rate dependent on the recruitment of neutrophils to the infection site. Neutrophils from naïve mice respond to CXC chemokines, but are usually unresponsive to CC chemokines. However, it has been showed that chemokine receptors expression profile can be alterated under sepsis conditions. Data from our laboratory show that CXCR2 expression is down regulated, impairing the neutrophil migration to infection focus. In addition, CCR2 appears on the surface of neutrophils, mediating the accumulation of these cells in the lung and other organs. In this context, we aimed to investigate the possible expression of CCR5 receptor on neutrophils and its role on sepsis evolution. **Methods and Results:** All experiments were performed according to our institution's ethical guidelines (n^0 169/2011). C57BL/6 and CCR5 deficient mice ($CCR5^{-/-}$) were used to induce sepsis using cecal ligation and puncture (CLP) model. We showed by flow cytometry that neutrophils from naïve C57BL/6 mice express high levels of CXCR2 and low levels of CCR5. However, during experimental sepsis, induced by CLP (n = 5), in parallel with CXCR2 internalization (Sham:69.2±4.1 and CLP:15.4±6.8%), neutrophils from the circulation (Sham:1.6±0.1 and CLP:41.7±7.6%) or from the peritoneal cavity (Sham:10.1±2.7 and CLP:20.3±2.3%) express higher levels of CCR5. Furthermore, neutrophils expressing CCR5 were negative for annexin 5, indicating that CCR5 expression is not related to neutrophil apoptosis during sepsis. Interestingly, $CCR5^{-/-}$ mice subject to CLP (n = 5) show decreased survival rate (WT:46% and $CCR5^{-/-}$:16%), reduction of neutrophil migration to the site of infection (WT:13.6±2.2 and $CCR5^{-/-}$:3.9±0.8x10^6cells), increase of neutrophil infiltration in lung (WT:10.6±0.6 and $CCR5^{-/-}$: 29.7±4.5 U/0.1g of lung) and increase levels of markers of injuries in heart (CK-MB-WT:458.8±65.3 and $CCR5^{-/-}$:994.4±30.1, U/L) and kidney (Urea-WT:58.5±11.6 and $CCR5^{-/-}$:158.0±21.1, mg/dL), when compared to wild type mice (WT). In addition, the incubation of bone marrow derived-neutrophils with LPS enhances the expression of CCR5 (RPMI:137.0±7.1 and LPS:234.0±9.32, MFI) and renders them responsive to CCL4 (a ligant of CCR5)-induced chemotaxis (RPMI:137.0±7.1 and LPS:234.0±9.3 cell/field). Moreover, we demonstrated that CCR5 receptor has an important role during neutrophil adhesion to the vascular endothelium before transmigration. **Conclusion:** In summary, we showed that CCR5 expression on neutrophils plays a host protective role, since $CCR5^{-/-}$ mice under sepsis conditions present reduced neutrophil migration to infection focus, high systemic inflammation and low survival rates. We also demonstrated that there is a switching of chemokines receptors expression on neutrophils during ongoing of sepsis: while CCR5 is expressed, CXCR2 is down-regulated. Thus, CCR5 expression may represent a host-protective compensatory mechanism that maintains neutrophil migration to infection focus impaired by the down-regulation of CXCR2. **Financial support:** CNPq, FAPESP, CAPES, FAEPA

04.074 Role of prophylactic antibiotic treatment in severe acute pancreatitis. Soares FS[1], Horewicz V[2], Menin A[2], Spiller F[1] [1]UFSC – Farmacologia, [2]UFSC – Microbiologia, Imunologia e Parasitologia

Introduction: Acute pancreatitis (AP) is a common pancreatic disease with an increasing incidence rate during the past two decades. Secondary infection of pancreatic necrosis has emerged as a major determinant for morbidity and mortality from acute pancreatitis. Bacterial contamination of pancreatic necrosis occurs in 40%–70% of patients with necrotizing pancreatitis and is associated with up to 80% of deaths from this disease. For several decades, administration of prophylactic antibiotics has been one of the greatest controversies worldwide about the management of severe acute pancreatitis. Therefore, the objective of the present study is evaluated the role of prophylactic antibiotic treatment in severe experimental acute pancreatitis. **Methods:** C57BL/6 (8–10 weeks old) specific pathogen–free mice were divided into the following groups: sham-operated (SH), SH+meropenem (SH+M), severe acute pancreatitis (SAP) and SAP+meropenem (SAP+M). Mice received twice daily intraperitoneal injection of meropenem (100 mg/kg) for 3 days and then were subjected to obstruction of the common biliopancreatic duct to induction of SAP or to SH. Animals were evaluated for survival rate for 10 days or killed 24h after surgery and samples were harvested to bacterial count in the blood, ascitic fluid and cecum, colon and small intestine faeces. Bacterial growth was evaluated in aerobical and anaerobical conditions and susceptibility to meropenem was determined (CEUA approved protocol PP00662). **Results:** Surprisingly, meropenem prophylactic treatment induced 100% of mortality rate in SAP group, whereas only 40% of SAP control mice died two days after surgery. Moreover, meropenem treatment of SAP mice increased the CFU content in blood, ascitic fluid and also in samples collected from cecum, colon and small intestine. The bacterial populations founded in SAP+M mice showed resistance against meropenem in a disc diffusion assay. Furthermore, meropenem even increased CFU counting on SH group, suggesting that the antibiotic per se modifies gut microbiota profile. Importantly, treatment of naïve mice with meropenem (100 mg/kg) for 7 days did not induced tissue injury. **Conclusions and perspectives:** Our results showed that prophylactic treatment with meropenem accelerated the mortality rate in experimental severe acute pancreatitis and suggest that this treatment modifies gut microbiota profile, which can helpful the growth of intestinal pathobionts. Additional experiments are in progress to show the mechanisms involved in this phenomenon. **References:** Jiang, K. et al., *World J Gastroenterol*, 2012. Fritz, S. et al. *Am J Surg*, 2010. Yao, L. et al., *Dig Surg*, 2010. **Financial support:** CNPq.

04.075 Periodontitis impairs acetylcholine-induced relaxation of rat mesenteric arteries. Jesus FN[1], Wenceslau CF[2], Couto GK[2], Costa SKP[1], Rossoni LV[2], Muscará MN[1] [1]ICB-USP – Farmacologia, [2]ICB-USP – Fisiologia e Biofísica

Introduction: During the last years it has become evident that periodontal disease has systemic consequences, mainly involving the cardiovascular system. We have already observed endothelial dysfunction and decreased contractile response to norepinephrine in aortic rings from rats with periodontitis. In this study, we thus decided to study the *In vitro* response of the resistance mesenteric artery obtained from rats with periodontitis. **Methods:** The experimental protocol was approved by the ICB/USP ethics committee (CEUA) (No. 170, book 2, page113/2011). Male Wistar rats (70-100 days; n = 3-4/group) were anesthetized with i.p. 80 mg/kg ketamine plus 16 mg/kg xylazine. Periodontitis was induced by placing a cotton ligature around both the left and right lower first molars (sham animals had the ligature immediately removed). Seven days after, the rats were killed under anesthesia and the mesenteric bed was removed for isolation of the third-branch artery. The vessels were mounted on a wire myograph in order to evaluate the *In vitro* response to KCl, acetylcholine (ACh), phenylephrine (Phe) and sodium nitroprusside (NPS). Mean potency (as pD2) and maximal response (Emax) response values from both groups were compared by the Student t- test. **Results:** No differences in diameter, KCl- or Phe-induced contraction tension, or NPS-induced relaxation were observed between the groups. However, Ach-induced relaxation was lower in the vessels obtained from the animals with periodontitis, both in terms of pD2 (7.59±0.14 vs. 7.02 ± 0.17) or Emax (95±2.12 vs. 90 ± 5.3 %; $P<0.05$). **Conclusions:** Bilateral ligature-induced periodontitis in rats results in decreased endothelium-dependent (but not independent) vasodilatation of mesenteric artery, thus becoming a potential cause for the late appearance of related diseases such as hypertension or atherosclerosis. **Financial support:** FAPESP and CAPES.

04.076 Selective TNF-alpha inhibition with infliximab prevents inflammation but not diarrhea in irinotecan-induced intestinal mucositis. Pereira VBM[1], Lima-Júnior RCP[1], Figueiredo AA[1], Leite CAVG[1], Wong DVT[1], Pereira STA[1], Aragão KS[1], Bem AXC[1], Oriá RB[2], Magalhães PJC[1], Brito GAC[2], Souza MHLP[1], Ribeiro RA[1] [1]UFC – Physiology and Pharmacology, [2]UFC – Morphology

Introduction: Intestinal mucositis (IM) is a limiting side effect of anticancer therapy with Irinotecan (IRI). Previous studies reported that TNF-alfa seems to be a key mediator in many inflammatory responses, such as IM, rheumatoid arthritis and colitis. We have shown that non-selective inhibitors of cytokines attenuate IRI-induced IM. Besides, there is a lack of information about the effect of selective cytokine target therapy on anticancer drug toxicity. Then, the aim was to evaluate the role of a selective TNF-alfa inhibitor, Infliximab, on IRI-induced IM. **Methods**: C57BL/6 mice (n = 6) were divided into groups: saline (5 mL/kg, i.p.); IRI (75 mg/kg /4 days, i.p); Infliximab (5 mg/kg , i.v)+IRI. Diarrhea was assessed daily. Animals were killed on day 5 and the duodenum was collected for myeloperoxydase (MPO, U/mg tissue), IL-1 beta dosage (pg/mL), western blot of the inducible nitric oxide synthase, *In vitro* duodenal contractility and white blood cell count (cells/mm3). Data were analyzed with ANOVA/Student Newman Keul or Kruskal Wallis/Dunn's test. $P<0.05$ was accepted. Ethics Committee 99/10. **Results:** IRI induced a significant ($p<0.05$) diarrhea (1[0-2]), increased MPO activity (16.5±1.7), IL-1 beta dosage (431.5±158), iNOS expression (1.2±0.3), intestinal contractility (144±25.6), and leukopenia (7267±1180) compared with saline (0[0-0], 6.3±1.2, 1.8±1.8, 0.03±0.02, 55.7±11.8, 11100±1412, respectively). These effects were abrogated ($p<0.05$) by Infliximab (MPO: 8.7±1.9, IL-1 beta: 72.6±23.9, and iNOS expression: 0.14±0.1) compared with IRI. However, gut dysfunction (diarrhea: 1[0-2], intestinal contractility: 154±47.3) were not affected by infliximab ($p>0.05$). In addition, Infliximab potentiated ($p<0.05$) IRI-induced leukopenia (4067±518). **Discussion**: Thus, we showed the prominent anti-inflammatory effect of the target therapy anti-TNFalfa on IRI-induced IM. However, it did not counteract intestinal dysfunction and even potentiated the leukopenia, which might limit its use together with cancer chemotherapy. **Support**: CNPq /FUNCAP/CAPES

04.077 Insulin resistance mediates the exacerbate airway inflammatory response in obese sensitized mice. Calixto MC[1], Lintomen L, André DM[1], Leiria LOS[1], Ferreira DS[1], Landgraf RG[2], Anhê GF[1], Antunes E[1] [1]Unicamp – Farmacologia, [2]Unifesp – Ciências Biológicas

Introduction: There is accumulating evidence that obesity is associated with an increased risk of asthma. It has been hypothesized that insulin resistance, the hallmark of obesity and type II diabetes, plays a role in the development of asthma and thereby partly explain the association of asthma with obesity (Thuesen, BH, et al, *Clin Exp Allergy*.;39:700, 2009). Diet-induced obesity in mice stimulates eosinophilopoesis and enhances eosinophil (EOS) trafficking from bone marrow (BM) to lung tissues (Calixto, MC et al, *Br J Pharmacol*, 159:617, 2010). Communications between lung and BM play an important role in the pathogenesis of allergen-induced asthmatic responses. Therefore, the present study aimed to investigate the role of insulin resistance on EOS recruitment from BM to lung tissue in obese mice. **Methods:** The experimental protocols were approved by the Ethics Comitte of University of Campinas N°1496-1. Male C57bl6/J received a high-fat diet for 10 weeks. On the 8th week, animals were sensitized with OVA (100 µg, s.c.). Metformin treatment (300 µg/g/day/gavage) was given simultaneously to sensitization. Two weeks thereafter, mice were challenged with OVA (10 µg), after which EOS counts, production of cytokines and nitric oxide metabolites, inducible NO synthase (iNOS) expression, adhesion assays and flow cytometry were evaluated. **Results:** Metformin treatment significantly reduced the resistance to insulin action displayed by obese mice ($p<0.005$). Following OVA challenge, obese mice treated with metformin presented a decrease in the number of total inflammatory cells (45.18%) and EOS (42.17%) infiltrated into the lung tissue. The EOS rise by metformin in lung tissue was accompanied by decreases in TNF-α (72%), eotaxin (51%) levels and NOx (42%) in BALF compared with obese untreated, which in turn was accompanied by decreased expression of iNOS (43%) and p-IkB (47%) in lung. The metformin action on EOS recruitment from BM and lung infiltration was confirmed by a complete inhibition of TNF-α action through treatment with TNF-α antibody. To further examine the role of systemic insulin resistance in airways EOS recruitment, we analyzed the effects of metformin on EOS mobilization in BM of obese mice and underlying mechanisms for its migration to the inflammatory site. In obese mice, OVA challenge largely increased the BM EOS counts compared with lean group (2.2 ± 0.4 and $0.6 \pm 0.2 \times 10^6$/ml, respectively). Expression of VLA-4 e Mac-1 in BM EOS from obese mice was significantly lower ($P<0.05$) compared with lean animals, as assessed by flow cytometry assays. In addition, the adhesion of BM EOS to ICAM-1 and VCAM-1-coated plates *In vitro* was significantly lower (51% and 43%) in obese compared with lean mice. Metformin treatment was able to restore all the alterations observed in BM from obese challenge mice when compared to untreated mice. Our findings show that peripheral insulin resistance resulting from obesity accounts for the exacerbation of the lung inflammatory response in obese mice. Improving insulin resistance with metformin treatment led to normalization of eosinophil trafficking from BM into the airways lumen. **Financial support**: Fapesp

04.078 Lack of effect on MAP kinase phosphatase-1 expression underlies dexamethasone refractoriness in a murine model of asthma. Pão CRR, Serra MF, Cotias AC, Daleprane JB, Jurgilas PB, Couto GC, Anjos-Valotta EA, Cordeiro RSB[1], Silva PMR[1], Martins MA[1] [1]Fiocruz – Fisiologia e Farmacodinâmica

Introduction: Although glucocorticoids (GCs) are highly effective anti-inflammatory agents in asthma therapy, a small subgroup of patients shows persistent tissue inflammation and hyperreactivity of the airways (AHR) despite treatment with high doses of GC. Prior studies revealed that high levels of IL-2, IL-4 and IL-13 in the airways of patients with GC-resistant asthma reduce GC receptor nuclear translocation, by a mechanism dependent on p38MAPK (p38) phosphorylation. Notably MAP kinase phosphatase-1 (MKP-1) expression is upregulated by GC and exerts a pivotal role as a negative regulator of p38 activity. We have investigated here the effectiveness of the GC therapy on Th2 cytokines and p38/MKP-1 activity in a short-term murine model of asthma, marked by resistance to steroid therapy. **Methods:** Mice of strain A/J were subcutaneously sensitized on days 0 and 14 by a mixture of $Al(OH)_3$ and ovalbumin (OVA), and challenged for 2 (2-d) or 4 (4-d) consecutive days, starting at day 19 post-sensitization. Animals were treated with dexamethasone (DEX, 3 mg/kg , oral), or vehicle, 1 h before each provocation. ELISA quantified eotaxin-1, IL-4 and IL-13) in lung tissue samples. Western blotting was used to investigate p38 activity and MKP-1 expression. AHR was assessed by invasive plethysmography and eosinophilic infiltrate and peribronchial fibrosis were analyzed by histomorphometry. License CEUA number for this study is L-034/09. **Results and Discussion:** We found that A/J mice reacted to 2 consecutive daily i.n. allergen provocations with marked AHR, lung eosinophilia and subepithelial fibrosis. These changes were exacerbated in those animals subjected to the 4-d protocol of allergen provocations. DEX clearly inhibited allergen-evoked AHR, eosinophilia and peribronchial fibrosis noted in the 2-d regime, but failed to alter these changes in the 4-d regime. Similarly, increased generation of IL-4, IL-13 and eotaxin-1 were sensitive to DEX following 2, but not 4 consecutive allergen provocations. Remarkably, DEX reduced the levels of phosphorylated p38 MAPK (93%) in animals of the 2-d regime, leaving unchanged p38 MAPK in those animals of the 4-d regime. In parallel, there were elevations of MKP-1 expression of 3.2-fold and 1.8-fold, respectively. These findings show that A/J mice develop asthma-like pathological changes that are progressively exacerbated by the successive allergen provocations, becoming resistant to the steroid treatment as the magnitude of the allergic response increased. They also support the interpretation that changes in p38/MKP-1 regulation contribute to GC resistance in this model. **Financial support:** FAPERJ, CNPq and CAPES.

04.079 The effect of *Aedes aegypti* salivary gland on immune response induced by viral particles in model *in vitro*. Gomes RS, Navegantes KC, Monteiro MC UFPA – Farmácia

Introduction: Dengue, transmitted horizontally by arthropods such as *Aedes aegypti*, can be facilitated by the action of the mosquito's saliva. Participation of vector saliva in disease transmission has long been recognized. Saliva inoculated during blood feeding modulates the immune response allowing the infection to become established. On the other hand, anti-vector saliva immunity may protect the host against some vector-borne diseases Studies with other hematophagous reveal that these diseases may be favored by the actions anti-hemostatic and immunomodulatory properties of saliva, by regulating the host immune response potentiating infectious events. Thus, this study aimed to evaluate the immunomodulatory effect of the saliva of *Aedes aegypti* in dengue virus infection *In vitro.* **Methods:** The viral antigen DENV 2 and the saliva of *Aedes aegypti*, were provided, gently, by Instituto Evandro Chagas and Universidade Federal de Ciências da Saúde de Porto Alegre. In all tests were used spleen cells from BALB / c mice at a density of $2x10^5$ cells / ml. Cell viability was evaluated by using trypan blue 0.4%, and for the cell proliferation essay, spleen cells were incubated with Con A (5µg/mL) or DENV 2 (5µg/mL) in presence of *Aedes aegypti* saliva (0.5 salivary gland) in 5% CO2 at 37 ° C for 24 or 48 hours. Ten micro liters of methylthiazoletetrazolium (5 mg MTT / ml) was added 4 hours before the determination of the proliferative index, each group was performed with three replications. In addition to the proliferation and for evaluation of dead cells was also performed by flow cytometry analysis, so, splenocytes were treated as described above and washed with FACS-PBS, centrifuged and resuspended in a solution of citrate buffer with propidium iodide (PI).The DNA content in each cell nucleus and forward scatter (FSC) and side scatter (SSC) were set to 104 cells were gated using CellQuest software (FACSCalibur Flow Cytometer). Values were expressed in proliferative index and percentage of dead cells. Statistical Analysis was used one-way ANOVA,* $p \leq 0.05$. **Results and Discussion:** In proliferation assay induced by viral particles for MTT, our results showed that after 24 hours, all stimulus (ConA and DENV) induced significant proliferation of spleen cells, whereas the saliva was able potentiate the proliferation induced by Con A and DENV2 (CON A = 1.45 ± 0.173; DENV = 1.65 ± 0.25, CON A+SV = 2.49 ± 0.37; DENV +SV = 3.43 ± 0.27). On the other hand, the flow cytometry analysis showed that the saliva had a antiproliferative action when incubated with DENV, but not with CON A (CON A = 69.5%; DENV = 51%, CONA+SV = 68.2%; DENV +SV = 41.6%) and the saliva protected spleen cells from death (CON A = 2.9%; DENV = 3.1%, CON A+SV = 0.5%; DENV+SV = 0.8%). After 48 hours, all stimulus also induced proliferation of spleen cells compared to control, however, the presence of saliva did not change the proliferation induced by DENV and CON A. Our finds agree that *Aedes* salivary gland suppressed cell proliferation and it may contribute to pathogen transmission. **Financial support:** CAPES/CNPq, FAPESPA-PA; UNIVERSAL/CNPq, UFPA.

04.080 Reactive oxygen species-dependent inflammasome activation mediates irinotecan-induced mucositis through the control of IL-1B and IL-18 release. Arifa RDN[1], Madeira MFM[1], De Paula TP[1], De lima RL[1], Fagundes CT[1], Tavares LD[1], Rachid MA[2], Riffel B[3], Teixeira MM[4], Souza DG[1] [1]UFMG – Microbiologia, [2]UFMG – Patologia, [3]Université d´Orléans / CNRS, [4]UFMG – Bioquímica

Introduction: Irinotecan is a chemotherapeutic utilized during treatment of several solids tumours. Therefore, treatment with irinotecan is associated with several side effects, as leucopenia, diarrhea and mucositis. In addition, during mucositis occurs released of cytokines and ROS. Recently, has been shown that ROS may promote inflammation by activating the inflammasomes leading to caspase-1 activation and subsequent cleavage of pro-IL-1β and pro-IL-18 cytokines into their mature forms. Interestingly, IL-1β is release during mucositis and treatment with the IL-1 receptor antagonist decreases the severity of mucositis. However the mechanisms involved in such protection and the pathways that mediate IL-1 production are still unknown. Thus, the aim of this study was to assess the role of inflammasome and of the molecules involved in the activation of this complex during Irinotecan-induced mucositis. **Methods**: Experimental intestinal mucositis in mice was induced with Irinotecan (75 mg/kg) administered intraperitoneally (i.p.), once a day, for four consecutive days. It was utilized mice WT, $ASC^{-/-}$, $ICE^{-/-}$, $IL\text{-}18^{-/-}$,$GP91phox^{-/-}$. Seven days after the beginning of the Irinotecan treatment, mice were euthanized by cervical dislocation. For the evaluation of the role of IL-1 receptor, WT or *IL-18*$^{-/-}$ mice were treated subcutaneously (s.c.) with IL-1Ra (4 mg/kg) or saline, 8 hours before the first dose of Irinotecan. Then, mice were treated every 8 hours for the following 7 days, during all experimental period. For NOX inhibition, WT mice received one i.p injection of Apocynin (20 mg/kg) 24 hours before the first dose of Irinotecan and then a daily i.p. injection of Apocynin along all the experimental period. Ileum was removed to analyze of citokynes (ELISA), influx of neutrophils (MPO), burst oxidative (TBARS and GSH), analyze histhological and Western Blotting. **Results and conclusion**: Mice $ASC^{-/-}$ and $ICE^{-/-}$ presented less intestinal lesions, less MPO and less production of IL-1β and IL-18. In addition, mice *IL-18*$^{-/-}$ and WT mice treated with IL-1Ra was characterized by less MPO and less intestinal lesion. In addition, treatment with IL-1ra decreases the levels of IL-18 in WT mice and *IL-18*$^{-/-}$ mice presented less production of IL-1β.The animals WT that received Irinotecan showed increase TBARS and increase of the expression of caspases-1 , while *gp91*$^{phox-/-}$ or Apocynin-treated mice presented decreased this parameters (It was considered differences between groups when $p<0,05$). These results demonstrate the participation of inflammasome in the development of Irinotecan-induced mucositis and show that ROS derived from NADPH oxidase (NOX) control caspase-1 activation during mucositis, since absence of $gp91^{phox}$, a component of NOX, or pharmacological inhibition of this enzyme resulted in reduced cleavage of caspase-1 and protection of intestinal injury caused by Irinotecan.
Approved by CETEA , protocol 113/11. Supported by CNPq

04.081 Effects of caffeinated and decaffeinated coffee in the inflammatory alterations associated to obesity in mice. Caria CRP, Acedo SC, Rocha T, Gambero A UFS – Clinical Pharmacology and Gastroenterology

Aims: Previous studies have found that coffee drinking was protective against type 2 diabetes and recently, it was observed that caffeine can protect against non-alcoholic fatty liver disease (NAFLD), pathologies associated to obesity. Thus, we evaluated the effects of caffeinated and decaffeinated coffee in metabolic and inflammatory alterations associated to obesity using a model of diet induced obesity in mice.
Methods and Results: Swiss mice were feed with commercial chow (CN) or high fat diet (HFD) during 12 weeks (n = 12/group) and treated with caffeinated and decaffeinated coffee prepared by infusion of 1.5g of coffeein 300 ml of hot water during the last 2 weeks *ad libitum*. Body weight, food intake, glucose blood levels and insulin tolerance test (ITT) were evaluated. Hepatic and adipose tissue cytokines level were evaluated by ELISA. Steatosis area was also evaluated in liver biopsies. There was no reduction in body weight, food intake (or adiposity in animals after coffee drinking. No changes in glucose blood levels and insulin sensibility was also observed between groups. In caffeinated HFD group was observed an increase in adiponectin levels in adipose tissue when compared to controls and decaffeinated coffee (108.3±11.9, 133.6±13.9 and 161.3±13.3 ng/ml for control, decaffeinated and caffeinated HFD group, respectively; $p<0.05$) and, IL-10 in adipose tissue when compared to controls and decaffeinated coffee (137.1±8.4, 149.1±19.5 and 196.4±40.9 ng/ml for control, decaffeinated and caffeinated HFD group, respectively) and liver (1335±42, 1229±57 and1832±35 ng/ml, respectively; $p<0.05$). Histological analysis revealed that there was a reduction in steatosis area in caffeinated and decaffeinated HFD group when compared to control HFD group(82±3, 59±4 and 59±9% of steatose area for control, decaffeinated and caffeinated HFD group, respectively; $p<0.05$). **Conclusion:** Two weeks of caffeinated coffee drinking did not promote improvements in metabolic parameters or weight gain in mice fed with HFD. But, the levels of anti-inflammatory cytokines adiponectin and IL-10 were improved in adipose tissue and liver, suggesting that coffee consumption could positively influence the inflammatory alterations associated to obesity. Comitê de Ética é 001.10.11 **Financial support:** CAPES

04.082 Pharmacokinetics of ropivacaine in drug delivery systems. Papini JZB[1], Pinheiro M[1], Calafatti SA[1], Pedrazzoli J[1], Araújo DR[2], De Paula E[3], Cereda CMS[3], Tofoli GR[1] [1]Universidade São Francisco, [2]UFABC, [3]Unicamp

Ropivacaine (RVC) has been widely used in clinic practice. However, due to its quick redistribution and transference from the injection site it has short duration. The use of drug delivery systems, such as liposome, cyclodextrin and polymers with RVC, promotes slow drug release, prolonged anesthetic effect and reduced toxicity. Thus, the aim of this study was to evaluate the pharmacokinetic of new RVC formulations associated with liposome, cyclodextrin and polymers. The protocol was approved by the Institutional Committee for Ethics in Animal Research of São Francisco University (protocol #001.08.10). Twenty four rabbits were divided, into four groups (n = 6), which received the following treatments: RVC plain ($RVC_{0.5\%}$), RVC liposome-encapsulated ($RVC_{0.5\%LUV}$), RVC inclusion complex with 2-hidroxypropyl-beta-cyclodextrin ($RVC_{0.5\%CD}$) and associated with poloxamers ($RVC_{0.5\%POL}$) after sciatic nerve block (3ml). Blood samples (2 mL) from an ear vein were collected via a heparinised cannula pre dose (0 min) and at 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, 480 and 540 minutes after the injection of formulations. Immediately after each blood collection, plasma was separated and stored at −70°C until analysis. RVC plasma levels were determined using a Shimadzu LC 20 AD coupled to a Micromass Quattro Premier LC® triple stage quadrupole mass spectrometer (LC-MS/MS), equipped with an API electrospray source. Pharmacokinetics parameters were determined with WinNonlin program (version 5.3). Data were submitted to ANOVA/Tukey tests ($\alpha = 0.05$). In general, plasma levels obtained after the administration of different formulations ($RVC_{0.5\%LUV}$, $RVC_{0.5\%HP\text{-}\beta\text{-}CD}$ and $RVC_{0.5\%POL}$) did not show statistically significant differences when compared to $RVC_{0.5\%}$ injection. $RVC_{0.5\%POL}$ injection promoted lower concentrations when compared to $RVC_{0.5\%}$ after 15, 30 and 45 minutes ($p<0.05$). Pharmacokinetic analysis showed that the maximum plasma concentration (Cmax) after $RVC_{0.5\%POL}$ injection was smaller ($p<0.05$) when compared to $RVC_{0.5\%}$. The others formulations presented no statistically significant differences in Cmax values ($p>0.05$). The half time of elimination (t ½), time to maximum plasma concentration (Tmax) and the areas under the curves ($AUC_{0\text{-}540}$ and $AUC_{0\text{-}\infty}$) presented no statistically significant differences ($p>0.05$) after the four treatments. Based on these results, we conclude that the association of RVC with poloxamers was able to modify the absorption of the anesthetic. Supported by FAPESP n°06/00121-9.

04.083 Pipecolyl xylidide, a non anesthetic analogue of bupivacaine, inhibits allergen-induced lung inflammation and airways hyperreactivity in a murine model of difficult to treat asthma. Cotias AC[1], Serra MF[1], Pão CRR[1], Couto GC[1], Olsen PC[1], Pires ALA[1], Costa JCS[2], Cordeiro RSB[1], Silva PMR[1], Martins MA[1] [1]Fiocruz – Fisiologia e Farmacodinâmica, [2]IOC

Introduction: The vast majority of asthmatics are controlled satisfactorily with regular inhaled glucocorticoids (GCs) with or without the addition of short- or long-acting bronchodilators. Unfortunately, a proportion of patients develop severe disease, which is relatively or totally refractory to glucocorticoid therapy. Several approaches have shown the efficacy of nebulized lidocaine in reducing the use of oral GCs in patients with moderate and severe asthma. We sought to study here the putative anti-asthma effect of pipecolyl xylidide (PIXY), a non-anesthetic bupivacaine precursor, on allergen-evoked lung inflammation and airways hyperreactivity (AHR) in a short-term A/J murine model of asthma marked by resistance to steroid therapy. **Methods:** Mice of strain A/J were subcutaneously sensitized on days 1 and 14 by a mixture of Al(OH)3 + ovalbumin (OVA) and daily challenged from days 19 to 22 by 25 µg OVA (25 µl, intranasal instillation), to establish a murine model of acute asthma characterized by airways inflammation and AHR. PIXY (0,25%, 0,5% or 1%) was aerosolized for 30 min 1 h before challenge. Dexamethasone (3 mg/kg , orally), used as referential anti-inflammatory agent, was administered 1 h before provocation. AHR was assessed by invasive plethysmography. Peribronchial eosinophil infiltration was measured by histomorphometry. Lung cytokines and chemokines were quantified by ELISA 24 h after the last provocation. Further *In vitro* studies with T cells were conducted by stimulating with ovalbumin T lymphocytes from DO11.10 mice, in the presence or absence of PIXY treatment. Apoptosis and proliferation were assessed by staining DNA with propidium iodide and analyzing Sub-G0 and S+G2 population through flow cytometry, respectively. License number for this study is L-034/09. **Results and Discussion:** We observed that allergen provocation robustly increased peribronquial eosinophil infiltration, AHR, eotaxin-1, IL-4 and IL-5 levels. The nebulized PIXY, but not dexamethasone, significantly inhibited these changes. In another setting of experiments, PIXY increased apoptosis (50 ±1 to 91 ± 1 (Mean ± SEM, n = 5) and decreased proliferation percentage of allergen stimulated T lymphocytes *In vitro* (36 ± 1 to 15 ± 2 (Mean ± SEM, n = 5). Our findings show that nebulized PIXY inhibits allergen-induced airway hyperresponsiveness, TH2 cytokine generation and lung eosinophilic inflammatory infiltrate in a murine model of asthma expressing a certain degree of refractoriness to glucocorticoid treatment. These effects were likely due to an inhibition of proliferative activity of T lymphocyte cell function and survival. Take together, these results reinforce the interpretation that PIXY deserve further investigation concerning a putative clinical application in the control of asthma. **Financial Support:** FAPERJ, CNPq, PDTIS and CAPES

04.084 ß-caryophyllene, **a CB2 receptor agonist**, **ameliorates cyclophosphamide induced cystitis in rats**. Dornelles FN, Andrade EL, Bento AF, Calixto JB UFSC – Depto de Farmacologia

Introduction: Hemorrhagic cystitis is a common side effect observed in patients under chemotherapy with cyclophosphamide (CYP). Bladder inflammation is associated with alterations in neurochemical, electrophysiological, organizational and functional properties of micturition pathways. Recent evidence suggests that cannabinoid receptors are expressed in the urinary bladder and may affect bladder function (Merriam *et al*., 2008). β-Caryophyllene (BCP) is a plant volatile commonly found in the essential oils of numerous spice and food plants. BCP is also a major component in the essential oil of *Cannabis sativa* and selectively binds to the CB2 cannabinoid receptor, leading to cellular activation and anti-inflammatory effects (Gertsch *et al*., 2008). This study analyzed the anti-inflammatory and the antinociceptive effects of BCP in the rat model of CYP-induced cystitis. **Methods:** Male Wistar rats were used (200–220 g) and cystitis was induced by a single injection of CYP (200 mg/kg , ip). Animals were orally treated by gavage with 12.5, 25, or 50 mg/kg of BCP one hour before CYP injection. Breathing rate, closing of the eyes, and specific posture were scored at different time points after cystitis induction as nociception indexes. As inflammatory parameters, hemorrhage presence, edema formation, and bladder wet weight were determined macroscopically at 24 h after CYP administration. The mechanical hypernociception was measured with Von Frey filaments in the abdomen area and in the rat paw. All the experimental procedures were approved by the Animal Ethics Committees of Universidade Federal de Santa Catarina (protocol number PP00608). **Results:** The pre-administration of BCP in three different doses (12.5, 25, or 50 mg/kg , p.o) was used to determine the potential dose-dependent effects of BCP. At the two higher doses, 25 and 50 mg/kg , BCP resulted in a significant reduction (24.1±1.2% and 56.40±1.7%, respectively) of the behavioral score induced by CYP. Interestingly, BCP oral treatment (25 and 50 mg/kg , p.o) markedly attenuated the hemorrhage (37.6±12%, 50±12%) and the edema (37.6±12%, 50±12%) formation after CYP administration. Of great relevance, the higher dose of BCP (50 mg/kg , p.o) markedly reduced the bladder wet weight (26.7±5.8%). Of note, treatment with BCP (25 and 50 mg/kg , p.o) significantly attenuated the nociceptive responses induced by CYP on the rat paw (74.5±10%, 83.5±5.3%) and in the abdomen area (45.8±12%, 66.6±9.3%). **Conclusion:** In conclusion, the present results demonstrate that the plant-derived sesquiterpene BCP, given orally, exhibits preventive effects in CYP-induced cystitis. BCP consistently ameliorated the inflammatory and nociceptive signs associated with CYP- induced cystitis. Taken together, the present findings suggest that BCP could constitute an important alternative to prevent inflammation and nociception following chemotherapy with CYP. **Financial support:** Capes, CNPq. **References:** Merriam FV; et al. Neurosci Lett, 445(1): 130. 2008. Gertsch J; et al. PNAS, 105 (26): 9099. 2008.

04.085 Severity of irinotecan-induced small intestinal mucositis is regulated by the TLR9 pathways. Avila TV[1], Arifa RDN[1], de Paula TP[1], Costa VV[1], Cisalpino D[1], Ferraz FO[2], Madeira MFM[1], Teixeira MM[2], Souza DG[1] [1]UFMG – Microbiologia, [2]UFMG – Imunologia

Introduction: Intestinal mucositis is a serious complication of cancer chemotherapy and radiotherapy; the mechanism of mucositis development is still not fully understood. However, there are some evidences suggesting that it is dependent of an initiation phase of cell injury followed by induction of proinflammatory cytokines, which then leads to tissue inflammation and further cell death. Intestinal microbes or host cell debris from died cells containing DNA can be recognized by receptors such as TLR9. Once activated, they could modulate the expression of several inflammatory components such as inflammasomes components and proinflammatory cytokines. Thus, this study aimed to define the role of TLR9 signalling pathways on the modulation of inflammatory components and on the progression of small intestinal damage in a model of irinotecan-induced mucositis. **Methods:** Male (7–10 weeks old) C57BL/6J wild-type (WT) or TLR9 deficient mice ($TLR9^{-/-}$) were bred and maintained at animal facilities of Fiocruz–BH/MG. For induction of experimental intestinal mucositis, Irinotecan (75 mg/kg) was administered intraperitoneally (i.p.) once a day for four consecutive days and saline was used as control. Mice were weighed throughout the experiment once a day. Seven days after the beginning of the Irinotecan treatment, mice were euthanized by cervical dislocation. Blood was collected and ileum was removed to evaluation of citokynes (ELISA), influx of neutrophils (MPO) and expression of mRNA of inflammasome components by RT-PCR. The experimental protocol was approved by the Committee on the Ethics of Animal Experiments of the Universidade Federal de Minas Gerais (CETEA/UFMG, 113/11). **Results and Discussion:** After 7 days of Irinotecan treatment, WT mice showed about 30% of weight loss, marked reduction (5±2 cm) in intestinal length, decreased number of leucocytes in blood (Irinotecan: $12±7x10^5$/mL; saline: $52±8x10^5$/mL), increased MPO (Irinotecan: 0,8±0,3 relative units; saline: 0,14±0,06 relative units) and increased levels of IL-1β in ileum (Irinotecan:615±77 pg/100 mg of tissue; saline: 363±86 pg/ 100 mg of tissue) when compared to saline-treated mice. However, $TLR9^{-/-}$ mice showed about 10% of weight loss and less reduction (0,8±0,5cm) of the intestinal length at day 7 when compared to saline-treated mice. Those conditions were followed by less decreased number of leucocytes in blood ($34,4±6x10^5$/mL) and basal levels of MPO (0,1±0,06 relative units) and IL-1β (352±57 pg/ 100 mg of tissue) in ileum when compared to WT mice. (Results are shown as means ± S.E.M. Differences were compared by using analysis of variance (ANOVA) followed by Student-Newman-Keuls post-hoc analyses and $p<0.05$ was considered significant). Therefore, IL-1β mRNA expression in ileum was increased in a similar manner in WT or $TLR9^{-/-}$ mice treated with Irinotecan. Caspase-1 mRNA expression in ileum was increased in WT mice but it was found decreased in $TLR9^{-/-}$ group. Thus, lacking of TLR9 ameliorates mucositis injury. Indeed, it does not compromise IL-1β mRNA expression in ileum; however, the absence of TLR9 impairs the intestinal ability to produce caspase-1 and subsequent IL-1β maturation, attenuating the severity of irinotecan-induced small intestinal mucositis. **Financial support:** CAPES, CNPq and FAPEMIG

04.086 Dominant-negative inhibitor of soluble TNF XPro 1595 suppresses experimental silicosis in mice. Ciambarella BT[1], Arantes ACS[1], Trentin PG[1], Szymkowski DE[2], Martins MA[1], Silva PMR[1] [1]Fiocruz – Fisiologia e Farmacodinâmica, [2]Xencor

Introduction: Tumor necrosis factor (TNF) is a multifunctional cytokine known to regulate inflammation, which is presented on the cell surface as transmembrane TNF (tmTNF), acting to promote juxtacrine signaling, and soluble (solTNF), acting in a paracrine fashion. We demonstrated that mice depleted from TNFR1 gene and treatment with thalidomide markedly inhibited lung function alterations and tissue fibrosis in mice stimulated with silica particles, indicating that TNF plays an important role in such process. A previous report described the invention of a novel class of anti-TNF biologics (DN-TNFs), which selective inhibits the soluble form of TNF (Zalevsky J, J. Immunol, 179, 1872, 2007). In this study, we aimed to investigate the effect of the XPro 1595 on the experimental model of silicosis in mice. The monoclonal antibody against TNF infliximab was used for comparison. **Methods:** Male Swiss-Webster mice were intranasally instilled with 10 mg/50uL of silica particles, and then treated therapeutically with XPro 1595 (1.25 and 10 mg/kg, i.p.) and infliximab (1.25 mg/kg) on days 7, 14 and 21 post-silica. The analyses were performed 24 h after the last dose and included the following parameters: i) lung function and airways hyperreactivity to methacholine (invasive plethysmography -Finepoint Buxco System), ii) morphology and morphometry (H&E and picrus sirius), iii) collagen deposition (Sircol method) and iv) chemokine/cytokine generation (ELISA). All experimental procedures were performed in accordance with guidelines of the Committee on Use of Laboratory Animals of FIOCRUZ (L-034/09). **Results:** We showed that stimulation with intranasal silica led to an increase in the basal levels of lung resistance and elastance as well to airways hyperreactivity to aerosolized methacholine, A marked tissue leukocyte infiltration accompanied by fibrosis (collagen deposition and granuloma formation) were also noted in the lungs silicotic mice. Increased levels of chemokines/cytokines (MIP-1a, MIP-2, IL-1β, TGF-β, KC and MCP-1) were also noted. Therapeutic administration of XPro 1595 and infliximab significantly inhibited the exacerbation of increase resistance and elastance as well as fibrosis, including increase in collagen deposition and granuloma formation in silica-stimulated mice. Chemokine and cytokine generation was also sentitive to XPro 1595 and infliximab. **Conclusion:** Altogether our findings show that treatment with XPro 1595 and infliximab effectively inhibited some critical features of silicosis, including alteration of lung function and fibrogenic response, reinforcing the idea that TNF is implicated in this disease. They also indicate that inflammation in mouse silicosis seems to be primarily driven by sol TNF. **Financial support:** FIOCRUZ/CNPq/FAPERJ.

04.087 PI3K, **ERK 1/2 and P38 pathways inhibited by hydrogen peroxide in the antigen-induced arthritis in mice**. Lopes F[1], Gonçalves W[1], Amaral F[2], Sousa LP[2], Teixeira M[2], Pinho V[1] [1]UFMG – Morfologia, [2]UFMG – Bioquímica

Introduction: Neutrophils are critical effector cells in innate immune responses and are the most abundant of all leukocytes in the joints of mice with antigen-induced arthritis (AIA). The neutrophil accumulation in tissue depends not only on the number of cells being recruited but also on the number of cells being cleared (by apoptosis) or leaving the tissue by transmigration. Many signal transduction pathways are important of survival of leukocytes in inflammatory sites, such as MAPKs and PI3K. Reactive oxygen species (ROS) are able to interfere in signal transduction pathways important to regulators of cell survival. In the present study, we examined the ability of ROS, mainly hydrogen peroxide, to interfere in MAPKs and PI3K and to resolve the inflammation associated to arthritis induced by antigen in mice. **Methods:** AIA was induced by the injection of methylated bovine serum albumin (mBSA) into the knee joint of pre-immunized mice. We investigated the kinetics of neutrophil accumulation in joint cavity and as well as the kinetics of MAPKs and PI3K. The role of hydrogen peroxide was investigated by the administration of SOD (0.3 mg/kg), as so as hydrogen peroxide administration (0,5 mM). Neutrophil apoptosis was evaluated by morphological aspects and activation of caspase by administration ZVAD. The activation of P38, ERK1/2 and PI3K was evaluated by administration of pathways inhibitors (SB203580, UO126 and LY294002). The hypernorciception was evaluated for increasing pressure test. (All procedures were approved by the Ethics Committee – CETEA 166/06 – UFMG). **Results:** The peak of neutrophil accumulation was observed at 24 hours and returned to baseline levels by 48 hours after challenge. Coincidently, it was observed a peak of activation of P38, ERK1/2 and PI3K in the AIA 24 hours after the challenge, paralleling with the beginning of the natural resolution of this model. The administration of SOD reduced the amount of neutrophils, associated with the increase of apoptotic cell numbers and caspase activation, and the reduced activation of P38, ERK1/2 and PI3K, wich were associated with attenuation of hypernorciception. **Discussion**: We propose here that the natural resolution in the AIA is ROS dependent. SOD and hydrogen peroxide administration cause: (a) reduction of neutrophils in the inflammatory site, (b) increase of apoptosis and (c) inhibition of signal transduction pathways important of survival. These events attenuated hypernociception and loss of function. This work was sponsored by CNPq and FAPEMIG.

04.088 Suppressive effect of the flavonoid quercetin on lung inflammation caused by silica particles in mice. Lima YOA, Ferreira TPT, Arantes ACS, Martins MA, Silva PMR IOC-FIOCRUZ – Inflammation

Introduction: Among the respiratory occupational diseases, silicosis is the most disabling one. This disease results from a chronic inflammatory process with granuloma formation generated in response to silica particle deposition in the lungs. There is no efficient treatment available for fibrotic diseases, which demands the search for effective therapies to control silicosis. Quercetin is a flavonoid found in a variety of fruits and vegetables, shown to have anti-inflammatory and antioxidant properties. Thus, the goal of this study was to investigate the potential effect of therapeutic administration of quercetin on the experimental model of silicosis in mice. **Methods:** Male Swiss-Webster mice were instilled with intranasal silica (10 mg/50 µL) and quercetin (2.5 – 10 mg/kg) was administrated once a day, during 7 days, starting 21 days post- silica. The analyses were performed 24 h after the last dose. First, the pulmonary function was tested by invasive plethysmography (FinePointe – Buxco System), which allowed recording values of lung resistance and elastance. Then, the lungs were perfused with saline + EDTA (20 mM), removed, fixed with Milloning solution and processed for histological analyses. Morphological and morphometrical analyses were performed in tissue samples stained with H&E. Quantification of cytokine generation was made by ELISA. All experimental procedures were approved by the Ethics Committee of Animal Use of FIOCRUZ (License 034/09). **Results:** We noted that silicotic mice exhibited a significant increase in basal levels of lung resistance and elastance as well as airways hyperreactivity to aerolization with methacholine, as compared to control mice. A marked inflammatory response in the lungs was also detected, characterized by leukocyte infiltration, intense collagen deposition and granuloma formation. We showed that the oral administration of quercetin had a significant therapeutic effect on the increased pulmonary resistance and elastance as well as on the airways hyperreactivity to methacholine in the silicotic mice. Quercetin reduced the inflammatory infiltrate, fibrotic response, granuloma formation and collagen deposition. In parallel, the levels of IL-1β were clearly suppressed in the lung tissue of silica-challenged mice treated with quercetin. **Conclusion**: Our results show that the therapeutic administration of quercetin was effective in inhibiting alterations noted in silicotic mice, including reduced lung function and tissue fibrosis, suggesting that this compound may be of potential beneficial effect for the treatment of fibrotic diseases. Additional experiments are underway to clarify better the mechanism of action involved in the suppressive effect of quercetin on silicosis. **Financial support:** FIOCRUZ, CNPq, FAPERJ.